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(57) Abstract

An adenovirus wherein at least one portion of at least one loop region of the hexon is changed. In one embodiment, the adenovirus, prior to modification, is of a first serotype, and at least a portion of at least one loop region of the hexon is removed and replaced with at least a portion of at least one loop region of the hexon of an adenovirus of a second serotype. Such modified adenoviruses do not have epitopes which are recognized by neutralizing antibodies to the unmodified adenovirus of the first serotype.

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ADENOVIRUSES HAVING ALTERED HEXON PROTEINS

This application is a continuation-in-part of applciation Serial No. 08/788,674, filed January 24, 1997, the contents of which are incorporated by reference in their entirety.

This invention relates to adenoviruses which may be employed in gene therapy. More particularly, this invention relates to adenoviruses in which a portion of the hexon protein of the adenovirus is changed. Still more particularly, this invention relates to adenoviruses wherein at least a portion of at least one loop region of the hexon is changed.

BACKGROUND OF THE INVENTION

Adenovirus genomes are linear, double-stranded DNA molecules of approximately 36 kilobase pairs. Each extremity of the viral genome has a short sequence known as the inverted terminal repeat (or ITR), which is necessary for viral replication. The well-characterized molecular genetics of adenovirus render it an advantageous vector for gene transfer. Portions of the viral genome can be substituted with DNA of foreign origin. In addition, recombinant adenoviruses are structurally stable.

Adenoviruses thus may be employed as delivery vehicles for introducing desired polynucleotide sequences into eukaryotic cells, whereby the adenovirus delivers such polynucleotide sequences to eukaryotic cells by binding cellular receptors.

Adenoviral vectors, however, elicit immune responses, and such immune responses correlate with decreased efficiency of gene transfer and expression after repeated administration. (Yei, *et al.*, Gene Therapy, Vol. 1, pgs. 192-200 (1994)). It also was found that neutralizing antibodies to adenovirus block successful repeat administration of the adenovirus. (Smith *et al.*, Nature Genetics, Vol. 5, pgs. 397-402 (1993); Kozarsky, *et al.*, J. Biol. Chem., Vol. 269, No. 18, pgs. 13695-13702 (May 1994)).

Immunity to adenovirus is type specific (Wadell, "Molecular Epidemiology of Human Adenoviruses," in Current Topics in Microbiology and Immunology, Vol. 110, pgs. 191-220 (1984)), and infection with a particular serotype of adenovirus confers immunity only to that serotype. Successful DNA transduction has been demonstrated using sequential administration of different serotypes. (Mastrangeli, *et al.*, Human Gene Therapy, Vol. 7, pgs. 79-87 (January 1, 1996)). In Mastrangeli, an immunizing dose of wild-type Adenovirus 5 (subgroup C), Adenovirus 4 (subgroup E), or Adenovirus 30 (subgroup D) was administered intratracheally to rats, followed by an intratracheal administration of a replication-deficient subgroup C-derived recombinant adenovirus. Efficient gene transfer was not achieved in the rats that were given Adenovirus 5. In contrast, effective gene transfer was achieved in the rats that were given Adenovirus 4 or Adenovirus 30.

Kass-Eisler, *et al.*, Gene Therapy, Vol. 3, pgs. 154-162 (1996) disclose the administration of a vector derived from Adenovirus 5 which includes a chloramphenicol transferase (CAT) gene to one-day-old mice. Sixty days later, the mice received a second dose of the same vector. After the second administration, expression of CAT increased from about 2,900 units at a 57-day time point to about 27,000 units five days after the second administration. Although the expression of CAT increased, increases in the levels of neutralizing antibodies against

Adenovirus 5 also were detected. Thus, Kass-Eisler, *et al.* show that a second injection of adenovirus is possible only if the normal immune response is "circumvented," such as, for example, by administering the first dose to neonatal mice that are incapable of mounting an effective immune response and perhaps become "tolerant" of the injected adenovirus.

SUMMARY OF THE INVENTION

The present invention is directed to adenoviruses having altered antigenic epitopes. More particularly, the present invention is directed to an adenovirus having an altered hexon protein, and still more particularly, the present invention is directed to an adenovirus of a particular serotype wherein at least a portion of at least one of the loop regions of the hexon of such adenovirus is removed and replaced with at least a portion of the loop region(s) of the hexon of an adenovirus of another serotype.

BRIEF DESCRIPTIONS OF THE DRAWINGS

This invention now will be described with respect to the drawings, wherein:

Figure 1A shows a computer alignment (DNASTAR MegAlign software) of the predicted hexon amino acid sequences based on published nucleotide sequences, of human Adenovirus 12 (shown as AD12.PRO) (Sprengel, *et al.*, J. Virol., Vol. 68, pgs. 379-389 (1994)) and human Adenovirus 5 (shown as AD5.PRO) (Kinloch, *et al.*, J. Biol. Chem., Vol. 259, pgs. 6431-6456 (1984)). Identical or similar (conservative change) amino acids are boxed. The positions of the loop regions L1, L2, and L4 are shown (based on comparison with the sequence of human Adenovirus 2 as published by Roberts, *et al.*, Science, Vol. 232, pgs. 1148-1151 (1986). The positions where the restriction enzymes AgeI, BsrGI, and BamHI cut the corresponding nucleotide sequences also are indicated.

Figure 1B shows a computer alignment (DNASTAR MegAlign software) of the published nucleotide sequences coding for the hexons of human Adenovirus 12 (shown as AD12.SEQ) and

human Adenovirus 5 (shown as AD5). Identical nucleotides are boxed. The recognition sequences for the restriction enzymes AgeI, BsrGI, and BamHI are shown. The regions corresponding to the sequences from which the PCR primers used for amplification of the DNA sequences are shown.

Figure 2 is a map of the genome of the adenovirus Ad dl327 showing the position of the hexon coding region. The positions of the loop regions L1, L2, and L4 are shown. The recognition sites for the restriction enzymes AscI, AgeI, BsrGI, and BamHI (used for hexon DNA cloning constructions) are indicated.

Figure 3 shows the maps of plasmids pNEB193 and pAscBam, and a gel showing miniprep DNAs cut with HindIII.

Figure 4A shows a map of plasmid pAB12, and a gel showing 12 miniprep DNAs digested with HindIII.

Figure 4B shows a gel obtained after PCR of the Adenovirus 12 L1 region (upper left), with the arrowhead indicating the position of the PCR product; a gel (upper right) showing putative pABL1T miniprep DNAs cut with HindIII (expected 5,305 and 2,249 and 941 bp bands, lanes 2 to 7 numbered left to right) and a map of plasmid pABL1T.

Figure 5 shows maps of the plasmids pCRScript Direct, pAscAscf, and pAscAscr; a gel showing a diagnostic digest of pCRScript Direct (lower left), and a gel showing putative pAscAsc mini-prep DNAs cut with XmnI (lower right).

Figure 6 shows a plasmid map of pAA12, and a gel showing a diagnostic digest of clone DNA of pAA12 digested with EcoRI and BamHI; EcoRI and FseI; EcoRV; AseI; and HindIII.

Figure 7 shows a plasmid map of pAAL1T, a gel showing minipreps of two pABL1T clones which were double digested with HindIII and BamHI (lower left), and a gel showing a diagnostic digest of clone DNA of pAAL1T digested with HindIII, AscI, and SmaI (lower right).

Figure 8 shows maps of Av3nBg and Av12nBg, a gel showing DNA prepared from 293 cells infected with putative recombinant plaques digested with AscI and EcoRI and

electrophoresed on a 0.7% agarose gel (upper right), and a Southern blot (lower right) of such gel probed with a radioactively labeled 6,199 base pair, DNA fragment derived from pAA12 extending from the PstI site at bp 2,677 to the PstI site at base pair 8,876.

Figure 9 shows maps of Av3nBg and Av13nBg, and a gel showing DNA prepared from 293 cells infected with putative recombinant plaques digested with HindIII and electrophoresed on a 0.7% agarose gel.

Figure 10 is a Western Blot comparing anti-hexon reactivities of anti-Adenovirus 12, anti-Adenovirus 5, and monoclonal anti-hexon antibodies to proteins from pure virus preparations of Adenovirus 5, Adenovirus 12, and the chimeric virus Av12nBg.

Figure 11. Transduction of liver cells of C57BL6 mice by Av12LacZ previously immunized with Adenovirus 5. Genomic DNA prepared from liver samples was digested with the restriction enzyme ClaI and subjected to Southern hybridization using a 2,256 bp ClaI DNA fragment from the β-galactosidase gene as a probe. Copy number controls represent Av1LacZ4 copies per diploid genome spiked into normal mouse genomic DNA. Approximate copy numbers of β-galactosidase DNA in the livers of the mice were determined by comparison with the copy number standards by a quantitative analysis of band intensities in the autoradiogram using NIH-Image software.

Figure 12. Liver samples also were stained for β-galactosidase activity using X-gal as described in Yang, et al., Gene Therapy, Vol. 3, pgs. 412-420 (1996). Representative microscopic sections show β-galactosidase activity in livers from naive mice injected with Av1LacZ4(A), or Av12LacZ(B), or mice previously immunized with Adenovirus 5, and then injected with Av1LacZ4(C) or Av12LacZ(D).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with an aspect of the present invention, there is provided an adenovirus wherein at least a portion of at least one loop region of the hexon is changed.

In one embodiment, the adenovirus, prior to modification, is of a first serotype, and at least a portion of at least one loop region of the hexon of the adenovirus is removed and replaced with at least a portion of at least one loop region of the hexon of an adenovirus of a second serotype. In another embodiment, all of at least one loop region of the hexon of the adenovirus of the first serotype is removed and replaced with at least one loop region of the hexon of an adenovirus of a second serotype.

In a preferred embodiment, the at least one portion of at least one loop region of the hexon of the adenovirus of the first serotype is (are) a portion(s) of a loop region(s) which includes an epitope(s) which is (are) recognized by a neutralizing antibody(ies) to the adenovirus of the first serotype. By removing such epitopes and replacing them with polypeptides which are not recognized by neutralizing antibodies to the adenovirus of the first serotype, one constructs an adenovirus which resists inactivation by the immune system of the host.

Although the scope of the present invention is not intended to be limited to any theoretical reasoning, Applicant has found that when one administers an adenovirus of a particular serotype to a host, such administration of the adenovirus elicits a neutralizing antibody(ies), which recognize an epitope(s) of the loop region(s) of the hexon. The neutralizing antibody(ies) is (are) serotype specific. By replacing the loop region(s) of the hexon with loop region(s) obtained from an adenovirus of a different serotype, the modified adenovirus, when administered to a host, will not be inactivated by the neutralizing antibody(ies) which specifically recognize the loop region(s) of the hexon of the adenovirus of the first serotype. Thus, the modified adenovirus, which preferably also includes at least one DNA sequence encoding a therapeutic agent, may be administered to the host without being inactivated by the immune system of the host. In accordance with the present invention, one may construct a

series or battery of adenoviruses having a variety of altered or chimeric hexon proteins. The construction of such a series or battery of adenoviruses thus enables the repeated administration of recombinant adenoviruses to a host, while avoiding inactivation of the adenoviruses by the immune system of the host upon each administration of a recombinant adenovirus.

In yet another embodiment, at least a portion of at least one of the L1 and L2 loop regions of the hexon of the adenovirus of the first serotype is removed and replaced with at least a portion of at least one of the L1 and L2 loop regions of the hexon of the adenovirus of the second serotype. In another embodiment, all of at least one of the L1 and L2 loop regions of the hexon of the adenovirus of the first serotype is removed and replaced with all of at least one of the L1 and L2 loop regions of the hexon of the adenovirus of the second serotype. In a further embodiment, at least a portion of each of the L1, L2, and L4 loop regions of the hexon of the adenovirus of the first serotype is removed and replaced with at least a portion of each of the L1, L2, and L4 loop regions of the hexon of the adenovirus of the second serotype. In yet another embodiment, the L1, L2, and L4 loop regions of the hexon of the adenovirus of the first serotype are removed and replaced with the L1, L2, and L4 loop regions of the hexon of the adenovirus of the second serotype. In one embodiment, the first and second serotypes are from different adenovirus serotype subgenera.

In general, the human adenovirus serotypes are divided into Subgenera A through F. Such subgenera are described further in Bailey, et al., Virology, Vol. 205, pgs. 438-452 (1994), the contents of which are herein incorporated by reference. Subgenus A includes Adenovirus 12, Adenovirus 18, and Adenovirus 31. Subgenus B includes Adenovirus 3, Adenovirus 7, Adenovirus 34, and Adenovirus 35. Subgenus C includes Adenovirus 1, Adenovirus 2, Adenovirus 5, and Adenovirus 6. Subgenus D includes Adenovirus 9, Adenovirus 10, Adenovirus 15, and Adenovirus 19. Subgenus E includes

Adenovirus 4. Subgenus F includes Adenovirus 40 and Adenovirus 41. In one embodiment, the adenovirus of the first serotype is an adenovirus of a serotype within Subgenus C, and the adenovirus of the second serotype is an adenovirus of a serotype within one of Subgenera A, B, D, E, or F, and preferably within one of Subgenera A or F. In another embodiment, the adenovirus of the first serotype is an adenovirus of a serotype selected from the group consisting of Adenovirus 2 and Adenovirus 5.

In general, it is preferred that the at least a portion of the loop region(s) of the hexon which is (are) removed from the adenovirus is (are) replaced with loop region(s) from an adenovirus of a serotype where there is minimal conservation of the loop region(s) of the hexons of the adenoviruses of the different serotypes. For example, at least a portion of at least one loop region of the hexon of Adenovirus 5 is removed and replaced with at least a portion of at least one loop region of the hexon of Adenovirus 12. Applicant has found unexpectedly, that although there is minimal conservation of the loop region(s) of the adenoviruses of the different serotypes, such as the pair(s) of adenovirus serotypes hereinabove described, one is able to construct and generate successfully adenoviruses wherein the adenovirus in general is from a first serotype, and the loop region(s) of the hexon is (are) removed and replaced with the loop region(s) of the hexon of an adenovirus of a second serotype wherein there is minimal conservation of the loop region(s) of the hexons of the adenoviruses of the different serotypes. It is to be understood, however, that the scope of the present invention is not to be limited to adenoviruses of any particular serotypes.

Such adenoviruses may be constructed from an adenoviral vector of a first serotype wherein DNA encoding at least a portion of at least one loop region of the hexon is removed and replaced with DNA encoding at least a portion of at least one loop region of the hexon of an adenovirus of a second serotype.

The adenovirus, in general, also includes at least one DNA sequence encoding a therapeutic agent. The term "therapeutic" is used in a generic sense and includes treating agents, prophylactic agents, and replacement agents.

DNA sequences encoding therapeutic agents include, but are not limited to, DNA sequences encoding tumor necrosis factor (TNF) genes, such as TNF-α; genes encoding interferons such as Interferon-α, Interferon-β, and Interferon-γ; genes encoding interleukins such as IL-1, IL-1β, and Interleukins 2 through 14; genes encoding GM-CSF; genes encoding ornithine transcarbamylase, or OTC; genes encoding adenosine deaminase, or ADA; genes which encode cellular growth factors, such as lymphokines, which are growth factors for lymphocytes; genes encoding epidermal growth factor (EGF), and keratinocyte growth factor (KGF); genes encoding soluble CD4; Factor VIII; Factor IX; cytochrome b; glucocerebrosidase; T-cell receptors; the LDL receptor, ApoE, ApoC, ApoAI and other genes involved in cholesterol transport and metabolism; the alpha-1 antitrypsin (α1AT) gene; the insulin gene; the hypoxanthine phosphoribosyl transferase gene; negative selective markers or "suicide" genes, such as viral thymidine kinase genes, such as the Herpes Simplex Virus thymidine kinase gene, the cytomegalovirus virus thymidine kinase gene, and the varicella-zoster virus thymidine kinase gene; Fc receptors for antigen-binding domains of antibodies, antisense sequences which inhibit viral replication, such as antisense sequences which inhibit replication of hepatitis B or hepatitis non-A non-B virus; antisense *c-myb* oligonucleotides; and antioxidants such as, but not limited to, manganese superoxide dismutase (Mn-SOD), catalase, copper-zinc-superoxide dismutase (CuZn-SOD), extracellular superoxide dismutase (EC-SOD), and glutathione reductase; tissue plasminogen activator (tPA); urinary plasminogen activator (urokinase); hirudin; the phenylalanine hydroxylase gene; nitric oxide synthetase; vasoactive peptides; angiogenic peptides; the dopamine gene; the

dystrophin gene; the β-globin gene; the α-globin gene; the HbA gene; protooncogenes such as the *ras*, *src*, and *bcl* genes; tumor-suppressor genes such as p53 and Rb; the heregulin-α protein gene, for treating breast, ovarian, gastric and endometrial cancers; monoclonal antibodies specific to epitopes contained within the β-chain of a T-cell antigen receptor; the multidrug resistance (MDR) gene; DNA sequences encoding ribozymes; antisense polynucleotides; genes encoding secretory peptides which act as competitive inhibitors of angiotensin converting enzyme, of vascular smooth muscle calcium channels, or of adrenergic receptors, and DNA sequences encoding enzymes which break down amyloid plaques within the central nervous system. It is to be understood, however, that the scope of the present invention is not to be limited to any particular therapeutic agent.

The DNA sequence which encodes the therapeutic agent may be genomic DNA or may be a cDNA sequence. The DNA sequence also may be the native DNA sequence or an allelic variant thereof. The term "allelic variant" as used herein means that the allelic variant is an alternative form of the native DNA sequence which may have a substitution, deletion, or addition of one or more nucleotides, which does not alter substantially the function of the encoded protein or polypeptide or fragment or derivative thereof. In one embodiment, the DNA sequence may further include a leader sequence or portion thereof, a secretory signal or portion thereof and/or may further include a trailer sequence or portion thereof.

The DNA sequence encoding at least one therapeutic agent is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; and the ApoAI promoter.

It is to be understood, however, that the scope of the present invention is not to be limited to specific foreign genes or promoters.

The adenoviral vector which is employed may, in one embodiment, be an adenoviral vector which includes essentially the complete adenoviral genome (Shenk *et al.*, Curr. Top. Microbiol. Immunol., 111(3): 1-39 (1984). Alternatively, the adenoviral vector may be a modified adenoviral vector in which at least a portion of the adenoviral genome has been deleted.

In a preferred embodiment, the adenoviral vector comprises an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; a DNA sequence encoding a therapeutic agent; and a promoter controlling the DNA sequence encoding a therapeutic agent. The vector is free of at least the majority of adenoviral E1 and E3 DNA sequences, but is not free of all of the E2 and E4 DNA sequences, and DNA sequences encoding adenoviral proteins promoted by the adenoviral major late promoter.

In one embodiment, the vector also is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences.

In another embodiment, the vector is free of at least the majority of the adenoviral E1 and E3 DNA sequences, and is free of a portion of the other of the E2 and E4 DNA sequences.

In still another embodiment, the gene in the E2a region that encodes the 72 kilodalton binding protein is mutated to produce a temperature sensitive protein that is active at 32°C, the temperature at which the viral particles are produced. This temperature sensitive mutant is described in Ensinger *et al.*, J. Virology, 10:328-339 (1972), Van der Vliet *et al.*, J. Virology, 15:348-354 (1975), and Friefeld *et al.*, Virology, 124:380-389 (1983).

Such a vector, in a preferred embodiment, is constructed first by constructing, according to standard techniques, a shuttle plasmid which contains, beginning at

the 5' end, the "critical left end elements," which include an adenoviral 5' ITR, an adenoviral encapsidation signal, and an E1a enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a multiple cloning site (which may be as herein described); a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. The vector also may contain a tripartite leader sequence. The DNA segment corresponding to the adenoviral genome serves as a substrate for homologous recombination with a modified or mutated adenovirus, and such sequence may encompass, for example, a segment of the adenovirus 5 genome no longer than from base 3329 to base 6246 of the genome. The plasmid may also include a selectable marker and an origin of replication. The origin of replication may be a bacterial origin of replication. Representative examples of such shuttle plasmids include pAvS6, which is described in published PCT Application Nos. W094/23582, published October 27, 1994, and W095/09654, published April 13, 1995 and in U.S. Patent No. 5,543,328, issued August 6, 1996. The DNA sequence encoding a therapeutic agent then may be inserted into the multiple cloning site to produce a plasmid vector.

This construct is then used to produce an adenoviral vector. Homologous recombination is effected with a modified or mutated adenovirus in which at least the majority of the E1 and E3 adenoviral DNA sequences have been deleted. Such homologous recombination may be effected through co-transfection of the plasmid vector and the modified adenovirus into a helper cell line, such as 293 cells, by $CaPO_4$ precipitation. Upon such homologous recombination, a recombinant adenoviral vector is formed that includes DNA sequences derived from the shuttle plasmid between the Not I site and the homologous recombination fragment, and DNA derived from the E1 and E3 deleted adenovirus between the homologous recombination fragment and the 3' ITR.

In one embodiment, the homologous recombination fragment overlaps with nucleotides 3329 to 6246 of the adenovirus 5 (ATCC VR-5) genome.

Through such homologous recombination, a vector is formed which includes an adenoviral 5' ITR, an adenoviral encapsidation signal; an E1a enhancer sequence; a promoter; a DNA sequence encoding a therapeutic agent; a poly A signal; adenoviral DNA free of at least the majority of the E1 and E3 adenoviral DNA sequences; and an adenoviral 3' ITR. The vector also may include a tripartite leader sequence. The vector may then be transfected into a helper cell line, such as the 293 helper cell line (ATCC No. CRL1573), which will include the E1a and E1b DNA sequences, which are necessary for viral replication, and to generate adenoviral particles. Transfection may take place by electroporation, calcium phosphate precipitation, microinjection, or through proteoliposomes.

In another embodiment, the adenoviral vector is free of all or a portion of each of the adenoviral E1 and E4 DNA sequences, or is free of all or a portion of each of the adenoviral E1 and E2 DNA sequences, or is free of all or a portion of each of the E1, E2, and E4 DNA sequences.

Such vectors may be assembled by direct *in vitro* ligation from combinations of plasmids containing portions of modified or unmodified virus genome or plasmids and fragments derived directly from a linear adenoviral genome, such as the Adenovirus 5 genome (ATCC No. VR-5) or Adenovirus 5 derived viruses containing mutations or deletions.

In another alternative, the vectors can be assembled by homologous recombination, within a eukaryotic cell, between a plasmid clone containing a portion of the adenoviral genome (such as the Adenovirus 5 genome or the adenovirus 5 E3-mutant Ad dl327 (Thimmapaya, *et al.*, Cell, Vol. 31, pg. 543 (1983)) with the desired modifications, and a second plasmid (such as, for example pAvS6), containing the left adenoviral ITR, an E1 region deletion, and the desired trans gene. Alternatively, homologous

recombination may be carried out between a plasmid clone and a fragment derived directly from a linear adenovirus (such as Adenovirus 5, or Ad dl327 or an Adenovirus 5 derived virus containing mutations or deletions) genome.

The vector then is transfected into a cell line capable of complementing the function of any essential genes deleted from the viral vector, in order to generate infectious viral particles. The cell line in general is a cell line which is infectable and able to support adenovirus or adenoviral vector growth, provide for continued virus production in the presence of glucocorticoid hormones, and is responsive to glucocorticoid hormones (*i.e.*, the cell line is capable of expressing a glucocorticoid hormone receptor). Cell lines which may be transfected with the essential adenoviral genes, and thus may be employed for generating the infectious adenoviral particles include, but are not limited to, the A549, KB, and Hep-2 cell lines.

Because the expression of some viral genes may be toxic to cells, the E1 region, as well as the E2a, E2b, and/or E4 regions, may be under the control of an inducible promoter. Such inducible promoters may include, but are not limited to, the mouse mammary tumor virus (MMTV) promoter (Archer, *et al.*, Science, Vol. 255, pgs. 1573-1576 (March 20, 1992)); the synthetic minimal glucocorticoid response element promoter GRE5 (Mader, *et al.*, Proc. Nat. Acad. Sci., Vol. 90, pgs. 5603-5607 (June 1993)); or the tetracycline-responsive promoters (Gossen, *et al.*, Proc. Nat. Acad. Sci., Vol. 89, pgs. 5547-5551 (June 1992)). In another alternative, the E1 region is under the control of an inducible promoter, and the E2a, E2b and/or E4 regions are under the control of their native promoters. In such alternative, the native promoters are transactivated by expression of the E1 region.

In one embodiment, the cell line includes the entire adenoviral E4 region with its native promoter region, and the E1a region or the entire E1 region (including the E1a and E1b regions) under the control of a regulatable or

inducible promoter, such as, for example, the mouse mammary tumor virus (or MMTV) promoter, which is a hormone inducible promoter, or other such promoters containing glucocorticoid responsive elements (GRE's) for transcriptional control. In another embodiment, the E4 DNA sequence also is expressed from a regulatable promoter, such as the MMTV promoter. The E1 and E4 DNA sequences may be included in one expression vehicle, or may be included in separate expression vehicles. Preferably, the expression vehicles are plasmid vectors which integrate with the genome of the cell line.

Such vectors, wherein the vector is free of all or a portion of each of the adenoviral E1 and E4 DNA sequences, or is free of all or a portion of each of the adenoviral E1 and E2 DNA sequences, or is free of all or a portion of the E1, E2, and E4 DNA sequences, and the complementing cell lines, also are described in PCT Application No. WO96/18418, published June 20, 1996, the contents of which are incorporated herein by reference.

Upon formation of the adenoviral vectors hereinabove described, the genome of such a vector is modified such that DNA encoding at least a portion of at least one loop region os the hexon is removed and replaced with DNA encoding at least a portion of at least one loop region of the hexon of an adenovirus having a serotype different from that of the adenovirus being modified. Such modification may be accomplished through genetic engineering techniques known to those skilled in the art.

Upon modification of the genome of the adenoviral vector, the vector is transfected into an appropriate cell line for the generation of infectious adenoviral particles wherein at least a portion of at least one loop region of the hexon has been changed.

Alternatively, the DNA sequence encoding the modified hexon may be placed into an adenoviral shuttle plasmid such as those hereinabove described. The shuttle plasmid also may include a DNA sequence encoding a therapeutic agent. The shuttle plasmid is transfected into an appropriate cell

line for the generation of infectious viral particles, with an adenoviral genome wherein the DNA encoding the hexon is deleted.

In another alternative, a first shuttle plasmid includes the DNA sequence encoding the modified hexon, and a second shuttle plasmid includes a DNA sequence encoding a therapeutic agent. The shuttle plasmids are cotransfected into an appropriate cell line for the generation of infectious viral particles, with an adenoviral genome wherein the DNA encoding the hexon is deleted. Homologous recombination produces an adenoviral vector including a modified hexon protein.

The adenoviruses of the present invention may be administered to a host *in vivo* in an amount effective to provide a therapeutic effect in a host.

In one embodiment, the adenoviral vector may be administered in an amount of from 1 plaque forming unit to about 10^{14} plaque forming units, preferably from about 10^{6} plaque forming units to about 10^{13} plaque forming units. The host may be a mammalian host, including human or non-human primate hosts.

The infectious adenoviral vectors are administered to the lung when a disease or disorder of the lung (such as, for example, cystic fibrosis) is to be treated. Such administration may be, for example, by aerosolized inhalation or brochoscopic instillation, or via intranasal or intratracheal instillation.

In another embodiment, the infectious adenoviral vectors are administered systemically, such as, for example, by intravenous administration (such as, for example, portal vein injection or peripheral vein injection), intraarterial administration, intramuscular administration, intraperitoneal administration, intratracheal administration, or intranasal administration.

The adenoviral vectors may be administered in combination with a pharmaceutically acceptable carrier suitable for administration to a patient. The carrier may

be a liquid carrier (for example, a saline solution), or a solid carrier, such as, for example, microcarrier beads.

Cells which may be infected by the infectious adenoviral vectors include, but are not limited to, primary cells, such as primary nucleated blood cells, such as leukocytes, granulocytes, monocytes, macrophages, lymphocytes (including T-lymphocytes and B-lymphocytes), totipotent stem cells, and tumor infiltrating lymphocytes (TIL cells); bone marrow cells; endothelial cells; activated endothelial cells; epithelial cells; lung cells; keratinocytes; stem cells; hepatocytes, including hepatocyte precursor cells; fibroblasts; mesenchymal cells; mesothelial cells; parenchymal cells; vascular smooth muscle cells; brain cells and other neural cells; gut enterocytes; gut stem cells; and myoblasts.

The infected cells are useful in the treatment of a variety of diseases including but not limited to adenosine deaminase deficiency, sickle cell anemia, thalassemia, hemophilia A, hemophilia B, diabetes, α-antitrypsin deficiency, brain disorders such as Alzheimer's disease, phenylketonuria and other illnesses such as growth disorders and heart diseases, for example, those caused by alterations in the way cholesterol is metabolized and defects of the immune system.

In one embodiment, the adenoviral vectors may be used to infect lung cells, and such adenoviral vectors may include the CFTR gene, which is useful in the treatment of cystic fibrosis. In another embodiment, the adenoviral vector may include a gene(s) encoding a lung surfactant protein, such as SP-A, SP-B, or SP-C, whereby the adenoviral vector is employed to treat lung surfactant protein deficiency states.

In another embodiment, the adenoviral vectors may be used to infect liver cells, and such adenoviral vectors may include gene(s) encoding clotting factor(s), such as Factor VIII and Factor IX, which are useful in the treatment of hemophilia A and hemophilia B, respectively.

In another embodiment, the adenoviral vectors may be used to infect liver cells, and such adenoviral vectors may include gene(s) encoding polypeptides or proteins which are useful in prevention and therapy of an acquired or an inherited defect in hepatocyte (liver) function. For example, they can be used to correct an inherited deficiency of the low density lipoprotein (LDL) receptor, or a deficiency of ornithine transcarbamylase.

In another embodiment, the adenoviral vectors may be used to infect liver cells, whereby the adenoviral vectors include a gene encoding a therapeutic agent employed to treat acquired infectious diseases, such as diseases resulting from viral infection. For example, the infectious adenoviral vectors may be employed to treat viral hepatitis, particularly hepatitis B or non-A non-B hepatitis. For example, an infectious adenoviral vector containing a gene encoding an anti-sense gene could be employed to infect liver cells to inhibit viral replication. In this case, the infectious adenoviral vector, which includes a structural hepatitis gene in the reverse or opposite orientation, would be introduced into liver cells, resulting in production in the infected liver cells of an anti-sense gene capable of inactivating the hepatitis virus or its RNA transcripts. Alternatively, the liver cells may be infected with an infectious adenoviral vector which includes a gene which encodes a protein, such as, for example, α-interferon, which may confer resistance to the hepatitis virus.

In yet another embodiment, an adenoviral vector in accordance with the present invention may include a negative selective marker, or "suicide" gene, such as the Herpes Simplex Virus thymidine kinase (TK) gene. Such a vector may be employed in the treatment of tumors, including cancerous and non-malignant tumors, by administering the adenoviral vector to a patient, such as, for example, by direct injection of the adenoviral vector into the tumor, whereby the adenoviral vector transduces the tumor cells. After the cells are transduced with the

adenoviral vector, an interaction agent or prodrug, such as, for example, ganciclovir, is administered to the patient, whereby the transduced tumor cells are killed.

In another embodiment, the adenoviral vectors, which include at least one DNA sequence encoding a therapeutic agent, may be administered to an animal in order to use such animal as a model for studying a disease or disorder and the treatment thereof. For example, an adenoviral vector containing a DNA sequence encoding a therapeutic agent may be given to an animal which is deficient in such therapeutic agent. Subsequent to the administration of such vector containing the DNA sequence encoding the therapeutic agent, the animal is evaluated for expression of such therapeutic agent. From the results of such a study, one then may determine how such adenoviral vectors may be administered to human patients for the treatment of the disease or disorder associated with the deficiency of the therapeutic agent.

EXAMPLES

The invention now will be described with respect to the examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Construction of chimeric viruses based on Adenovirus 5 with hexons derived from Adenovirus 12

A. Cloning of Ad dl327 fragment into pNEB193

Ad dl327 (Thimmappaya, Cell, Vol. 31, pg. 543 (1983), incorporated herein by reference) is identical to Adenovirus 5 (Genbank Accession #M73260), except that an XbaI fragment including bases 28591 to 30474 (or map units 78.5 to 84.7) of the Adenovirus 5 genome, and which is located in the E3 region, has been deleted. A schematic of Ad dl327 is shown in Figure 2. Ad dl327 was cut with AscI and BamHI, and a fragment from base 15670 to base 21562, which includes the hexon of Adenovirus 5 was isolated. This fragment was cloned into pNEB193 (New England Biolabs) (Figure 3), which was cut with AscI and BamHI. Twelve miniprep DNA's of the resulting construct, cut with HindIII, were prepared. The minipreps were made using the boiling lysis method as described by Sambrook, *et al.*, Molecular Cloning, A Laboratory Manual, Vol. 1, pgs. 29-30, Cold Spring Harbor Laboratory Press (1989). About 500 ng of each DNA miniprep was digested with restriction enzymes and electrophoresed on agarose gels using standard procedures. One clone, clone number 11, pAscBam (Figure 3), was chosen for further experimentation.

B. PCR of Adenovirus 12 sequences and cloning of the amplified products

Figure 1A shows the alignment of the predicted hexon amino acid sequences (based on published nucleotide sequences) of human Adenovirus 12 and human Adenovirus 5. The alignment of Adenovirus 5 and Adenovirus 12 hexon (and flanking) nucleotide sequences are shown in Figure 1B. Because the AgeI recognition sequence (ACCGGT) is not preserved in Adenovirus 12, this sequence was synthesized

as a leader into one of the PCR primers used for this amplification (SZR46 - GCG ACC GGT CGC AGC GTC TGA CGC TGC GT). The BamHI site, however, is present in the Adenovirus 12 sequence. The downstream primer (SZR45 - GTG AAT GCG TAC CAC GTC G) that was synthesized was positioned downstream of the BamHI site (Figure 1B).

The PCR was carried out with the Elongase PCR Kit (Life Technologies, Inc., Gaithersburg, MD) exactly according to the manufacturer's instructions. The PCR mixture contained 50 ng of purified Adenovirus 12 DNA template, 200 μM of each deoxynucleoside triphosphate, 0.2 μM of each primer, Elongase buffer containing 1.6 mM Mg^{2+} and Elongase enzyme mixture in a 50 μl total volume. The samples were placed in a Perkin-Elmer thermocycler and subjected to PCR amplification as follows:

1. Pre-amplification denaturation: 94°C for 30 seconds;
2. Thermal cycling for 30 cycles: denaturation - 94°C for 30 seconds annealing - 55°C for 30 seconds; extension - 68°C for 7.5 minutes.
3. Hold at 4°C.

The 2,507 bp PCR product was double digested with AgeI and BamHI, and cloned in between the AgeI and BamHI sites of pAscBam to generate pAB12 (Figure 4A) DNA minipreps (numbered 8 through 18 from lane 3 as shown in Figure 4A, left to right) were digested with HindIII. All except clone number 14 showed the expected restriction pattern. Clones 12, 13, 15, and 16 were sequenced for about 300 bases from and including the upstream primer SZR46 hereinabove described. All four sequences were correct in and around the upstream primer. Clone 12 was used for further manipulations.

C. PCR of Adenovirus 12 sequence corresponding to most of the L1 loop (574 bp) and cloning of the amplified product

The primer SZR46 was synthesized as hereinabove described. The BsrGI site is located 5' to the end of the L1 region but is 3' to the most variable part of L1. The downstream primer (SZR57 - CGG TGT ACA ACA CAA CTT GAG CAG

TGT TTG C) was synthesized to incorporate and overlap the BsrGI site. (Figure 1B) This PCR was accomplished using Taq polymerase. The PCR mixture contained 50 ng of purified Adenovirus 12 DNA template, 200 μM of each deoxynucleoside triphosphate, 0.2 μM of each primer, Taq polymerase buffer (Boehringer Mannheim), and Taq polymerase enzyme in a 50 μl total volume. The samples were placed in a Perkin-Elmer thermocycler and subjected to PCR amplification as follows:

1. Pre-amplification denaturation: 95°C for 3 minutes.
2. Thermal cycling for 30 cycles: denaturation - 94°C for 30 seconds; annealing - 50°C for 30 seconds; extension - 72°C for 1 minute.
3. Hold at 4°C.

The PCR fragment was digested with AgeI and BsrGI and cloned in between these sites in pAscBam to generate pABL1T (Figure 4B). The gel shown in Figure 4B shows a digestion of six DNA minipreps with HindIII. All were correct. Clones 1 and 2 were chosen for further subcloning.

D. Cloning Ad dl327 DNA fragment into pCRScript Direct

Ad dl327 was cut with AscI, and the resulting DNA fragment from base 15670 to base 25290 was cloned into pCRScript Direct (Stratagene, La Jolla, California) that was linearized with AscI. Twelve DNA minipreps were checked by digestion with XmnI. Clone number 2 was determined to have a forward orientation, pAscAscf (Figure 5), and clone number 6 was determined to have a reverse orientation, pAscAscr (Figure 5).

E. Replacement of the Adenovirus 5 hexon sequences

pAscAscr was cut with FseI and BamHI and the 3,811 bp FseI-BamHI fragment of pAscAscr was replaced with the 3,714 bp FseI-BamHI fragment from pAB12 to generate pAA12 (Figure 6). The gel shown in Figure 6 shows a diagnostic digest of clone DNA with EcoRI and BamHI (lane 2 - fragments of 10,868 and 1,557 base pairs), EcoRI and FseI (lane 3 - fragments of 5,946 and 2,155 base pairs), EcoRV (lane 4 - fragments of 5,926, and 4,447, and 2,052 base pairs), AseI (lane 5 - fragments of 6,470, and 4,661, and 1,235, and 59

base pairs), and HindIII (lane 6 - fragments of 9,482, and 1,109, and 941, and 538, and 355 base pairs).

In another construction, the 3,811 bp FseI-BamHI fragment of pAscAscr was replaced with the 3,711 bp FseI-BamHI fragment from pABL1T to generate pAAL1T (Figure 7). Two clones (numbers 1 and 2 of pABL1T) were used as donors for the cloning. Six DNA minipreps were made from each cloning/transformation. The gel shown in Figure 7 (lower left) shows diagnostic digests of the minipreps. (Top row - 6 minipreps derived from pABL1T clone 1; bottom row - 6 minipreps derived from pABL1T clone 2). The DNAs were double digested with HindIII and BamHI (expected fragment sizes of 9,280, and 2,201, and 941 bp). The gel shown in Figure 7 (lower right) shows a diagnostic digest of the chosen clone DNA of pAAL1T digested with HindIII (lane 5, expected fragment sizes of 11,481 and 941 bp), AscI (lane 6, expected fragment sizes of 9,518 and 2,904 bp), and SmaI (lane 7, expected fragment sizes of 4,259, and 3,540, and 3,270, and 1,353 bp). Digests of pAscAscr with the same enzymes were run in lanes 1, 2, and 3 for comparison.

F. Replacement of the natural (wild type) Adenovirus 5 hexon in the adenovirus vector Av3nBg with the chimeric hexon constructs

The adenoviral vector Av3nBg is identical to the adenoviral vector Av3nLacZ, described in PCT Application No. WO96/18418, published June 20, 1996. Such vector has the genotype $E1^-$ $E2a^-$ $E3^-$ $E4^+$; i.e., such vector has deletions of the E1, E2a, and E3 DNA sequences.

Av3nBg DNA was digested with AscI, followed by destruction of AscI enzyme activity by digestion with Proteinase K.

pAA12 was digested with AscI, and a 9,521 bp fragment was gel purified. This fragment contains a chimeric hexon construct where the loop regions L1, L2, and L4 of Adenovirus 5 have been replaced with the L1, L2, and L4 loop regions of Adenovirus 12.

pAAL1T was digested with AscI, and a 9,518 bp fragment was gel purified. This fragment contains a chimeric hexon

construct where most of the L1 loop region of Adenovirus 5 hexon has been replaced with the L1 loop region from Adenovirus 12.

Each of the gel purified AscI fragments from pAA12 and from pAAL1T were ligated into the AscI digested Av3nBg DNA.

Each of the ligation products was transfected into 293 cells. Because Av3nBg DNA has a deletion of the E2a region which cannot be complemented in 293 cells, this transfection should select for recombinant adenovirus where the E2a function is present. Because the AscI fragments containing the chimeric hexon constructs contain an intact E2a region, this procedure exerts a biological selection for adenovirus recombinants containing the chimeric hexon.

G. Analysis of plaques

(i) Replacement of L1, L2, and L4 loop regions of Adenovirus 5 with homologous regions from Adenovirus 12

Five plaques were picked and amplified on 293 cells. DNA was isolated from the infected cells and digested with a combination of AscI and EcoRI, electrophoresed on a 0.7% agarose gel (Figure 8, upper right), and subjected to Southern blotting. The Southern blot was probed with a radioactively labeled probe made from a purified 6,199 bp fragment derived from pAA12, extending from the PstI site at base pair 2,677 to the PstI site at base pair 8,876. A map of Av3nBg showing the restriction sites for AscI and EcoRI, and a map of the desired recombinant virus (Av12nBg) are shown in Figure 8. The autoradiograph showing the result of the hybridization is shown in Figure 8 (lower right). The parent vector Av3nBg is expected to produce a hybridizing fragment having a size of 8,150 bp. The desired recombinant adenovirus, Av12nBg, with the chimeric hexon is expected to contain an EcoRI site within the hexon gene not present on the parent Adenovirus 5 hexon which would result in two hybridizing fragments of 5,285 and 4,236 base pairs, respectively. As seen in Figure 8, DNA from the expanded plaques 1, 2, 3, and 6 display the predicted hybridization pattern. DNA from plaque number 2 also appears to be free largely of contaminating DNA.

Therefore, plaque number 2 appears to be the desired recombinant adenovirus Av12nBg as shown in Figure 8. (The last lane on the right contains plasmid pAscAscr digested with AscI and EcoRI. The top band probably corresponds to incompletely digested plasmid DNA.) This recombinant virus was plaque purified, and a pure preparation of virus was made by employing a standard adenovirus purification protocol. The preparation was plaque titered. This preparation was used for antibody reactivity experiments.

(ii) Replacement of most of the L1 loop region with the homologous region from Adenovirus 12

Seven plaques (numbers 3, 4, 5, 6, 7, 8, and 9) were picked and amplified on 293 cells. DNA was isolated from the infected cells and digested with HindIII and electrophoresed on a 0.7% agarose gel along with Av3nBg DNA/HindIII and pAAL1T/HindIII (Figure 9). Plaque number 9 shows the presence of the 941 bp band expected from the desired adenovirus recombinant (Av13nBg).

Example 2

Testing chimeric viruses for reduced reactivity to antibodies to Adenovirus 5

A. Western blotting

About $2x10^9$ pfu of the virus Av12nBg was electrophoresed (in triplicate) alongside an equal amount of an Ad dl327 preparation and an Adenovirus 12 (ATCC No. VR-863) preparation. The sample buffer used for the electrophoresis contained 62.5mM Tris HCl, pH 6.8, 2% SDS, 1% glycerol, and 0.00125% bromophenol blue. The samples were not heated prior to electrophoresis. Under these conditions the hexon trimers do not separate into monomers. Following electrophoresis on a 4-15% polyacrylamide gradient gel, the separated proteins were electroblotted onto a PVDF membrane. The blot was cut into three identical strips, with each strip containing the three viruses being compared. The strips then were subjected to immunodetection using standard protocols. One strip was probed with a mouse monoclonal antibody (H467) with

reactivity to all hexon serotypes. The probe was used at a concentration of 0.2μg/ml. The two other strips were probed with serotype specific rabbit polyclonal antibodies to Adenovirus 5 (ATCC No. VR-1082 AS/Rab) and Adenovirus 12 (ATCC No. VR-1089 AS/Rab), respectively, at a 1:3,000 dilution. The Western Blot (Figure 10) shows that, as expected, the monoclonal antibody recognized the hexon trimer complex from all three virus preparations, and the anti-Adenovirus 5 and anti-Adenovirus 12 antibodies have preferential reactivities to their cognate hexons. The chimeric hexon was detected more readily by the anti-Adenovirus 12 antibody than the anti-Adenovirus 5 antibody.

B. Neutralization assays

The neutralization assays were conducted as described by Smith, et al., Nature Genetics, Vol. 5, pgs. 397-402 (1993) using Av1LacZ4 and Av12nBg as input indicator viruses. An equal amount (10^4 pfu) of each virus was incubated with serial dilutions of plasma from individual C57/B16 mice which had been injected previously with an Adenovirus 5 based adenovirus vector. Following the incubation, the virus was used to infect 293 cells in 96 well plates. The next day, the cells were stained for β-galactosidase expression by the indicator X-gal. In the absence of antibody, all the cells in the well showed β-galactosidase expression. The presence of neutralizing antibody in plasma is revealed by a reduction in the number of cells transduced by the indicator virus. The neutralization titer of each plasma was scored as the reciprocal of the dilution at which only about 25% of the cells in a well showed β-galactosidase expression. The results of three separate experiments are given in Tables I, II and III below.

Table I

	Neutralization titer (about 25% blue cells)	
Mouse	Av1LacZ4	Av12nBg

1	>1024	<8
2	>1024	<8
3	>1024	<8
4	>1024	<8
5	256	<8

Table II

	Neutralization titer (about 25% blue cells)	
Mouse	Av1LacZ4	Av12nBg
1	256	<2
2	8	<2
3	64	<2
4	256	<2
5	256	<2
6	1,024	<2

Table III

	Neutralization titer (about 25% blue cells)	
Mouse	Av1LacZ4	Av12nBg
1	>512	16
2	>512	<4
3	>512	<4
4	>512	<4

It was observed that most of the mice had high titer neutralizing antibodies against Av1LacZ4 as a result of their previous exposure to an Adenovirus 5 based vector. Importantly, only 1 out of 15 samples had a detectable, although low, neutralization titer against Av12nBg. These results show that neutralizing antibodies against Adenovirus 5 are less effective in neutralizing the new virus, Av12nBg, with the chimeric hexon.

Example 3

In order to confirm that mice immunized with Adenovirus 5 could be given the vector Av12LacZ, cohorts of three C57 BL/6 mice were immunized with a tail vein injection of 10^8 pfu of an Adenovirus 5 based vector, Av1ALAPH81, described in PCT Application No. WO94/29471, published December 22, 1994 and in Connelly, et al., Blood, Vol. 87, pgs. 4671-4677 (1996). The vector Av1ALAPH81 includes a B-domain deleted human Factor VIII gene. 10^8 pfu is a dose which previously had been determined to prevent re-administration. (Smith, et al., Gene Therapy, Vol. 3, pgs. 496-502 (1996)). After one month, each of the mice were challenged with $3x10^8$ pfu of either Av12LacZ or Av1LacZ4. Two days later, the mice were killed and the livers were analyzed for vector transduction by Southern blotting and by histochemical staining for β-galactosidase activity. The Souther Blot (Figure 11) showed that both Av1LacZ4 and Av12LacZ transduced the livers of naive mice efficiently. Only Av12LacZ, however, could transduce the livers of the mice which had been immunized by a previous administration of an Adenovirus 5 based vector. The results of the histochemical analysis (Figure 12) for β-galactosidase activity confirmed the Southern Blot data. Blue staining hepatocytes were seen with both vectors in naive mice, but only with Av12LacZ in mice immunized previously with the Adenovirus 5 based vector. Thus, the adenoviral vector including the chimeric hexon was efficacious *in vivo* in animals with circulating antibodies to Adenovirus 5.

The disclosures of all patents, publications (including published patent applications), database accession numbers, and depository accession numbers referenced in this specification are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication, database accession number, and depository accession number were specifically and individually indicated to be incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

WHAT IS CLAIMED IS:

1. A modified adenovirus wherein said adenovirus, prior to modification, is of a first serotype, said first serotype being within a first subgenus, and, wherein, in the modified adenovirus at least a portion of at least one loop region of the hexon of said adenovirus is removed and replaced with at least a portion of at least one loop region of the hexon of an adenovirus of a second serotype, said second serotype being within a second subgenus.

2. The adenovirus of Claim 1 wherein at least a portion of at least one of the L1 and L2 loop regions of the hexon of said adenovirus of said first serotype is removed and replaced with at least a portion of at least one of the L1 and L2 loop regions of the hexon of said adenovirus of said second serotype.

3. The adenovirus of Claim 2 wherein at least a portion of each of the L1, L2, and L4 loop regions of the hexon of said adenovirus of said first serotype is removed and replaced with at least a portion of each of the L1, L2, and L4 loop regions of the hexon of said adenovirus of said second serotype.

4. The adenovirus of Claim 3 wherein the L1, L2, and L4 loop regions of the hexon of said adenovirus of said first serotype are removed and replaced with the L1, L2, and L4 loop regions of the hexon of said adenovirus of said second serotype.

5. The adenovirus of Claim 1 wherein said adenovirus of said first serotype is an adenovirus of a serotype within Subgenus C, and said adenovirus of said second serotype is an adenovirus of a serotype within a subgenus selected from the group consisting of Subgenera A, B, D, E, and F.

6. The adenovirus of Claim 5 wherein said adenovirus of said second serotype is an adenovirus of a serotype within a subgenus selected from the group consisting of Subgenus A and Subgenus F.

7. The adenovirus of Claim 5 wherein said adenovirus of said first serotype is selected from the group consisting of Adenovirus 2 and Adenovirus 5.

8. The adenovirus of Claim 1 wherein said adenovirus of said first serotype is Adenovirus 5, and said adenovirus of said second serotype is Adenovirus 12.

9. The adenovirus of Claim 1 wherein said adenovirus further includes at least one DNA sequence encoding a heterologous protein.

10. A method of providing a therapeutic effect in a host, comprising:

administering to a host the adenovirus of Claim 9 in an amount effective to provide a therapeutic effect in a host.

FIG.1Aa	FIG.1Ab
FIG.1Ac	FIG.1Ad
FIG.1Ae	FIG.1Af

FIG.1A

FIG.1Ba	FIG.1Bb
FIG.1Bc	FIG.1Bd
FIG.1Be	FIG.1Bf
FIG.1Bg	FIG.1Bh
FIG.1Bj	FIG.1BK
FIG.1Bl	FIG.1Bm
FIG.1Bn	FIG.1Bo

FIG.1B

FIG. 1Aα

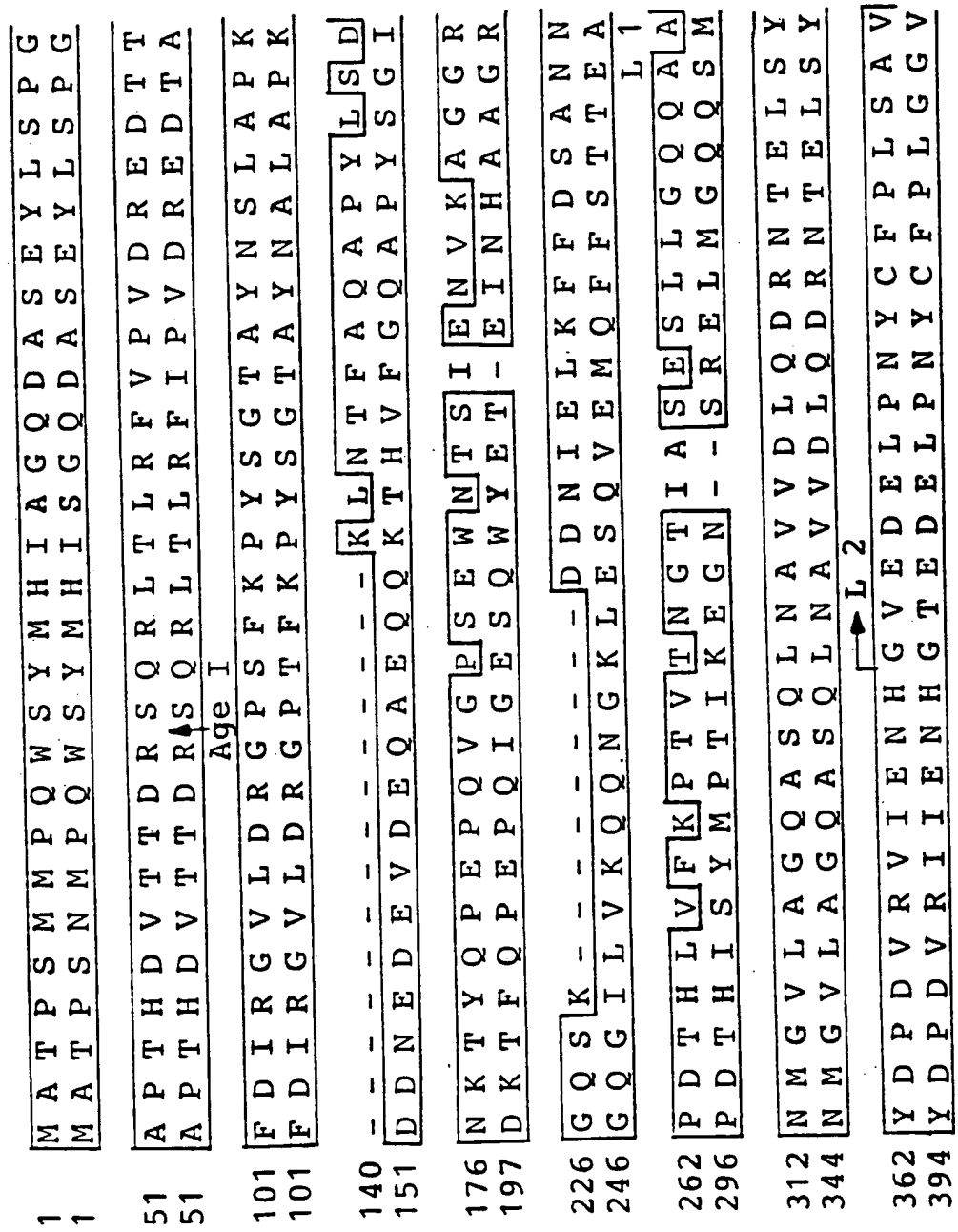

FIG. 1Ab

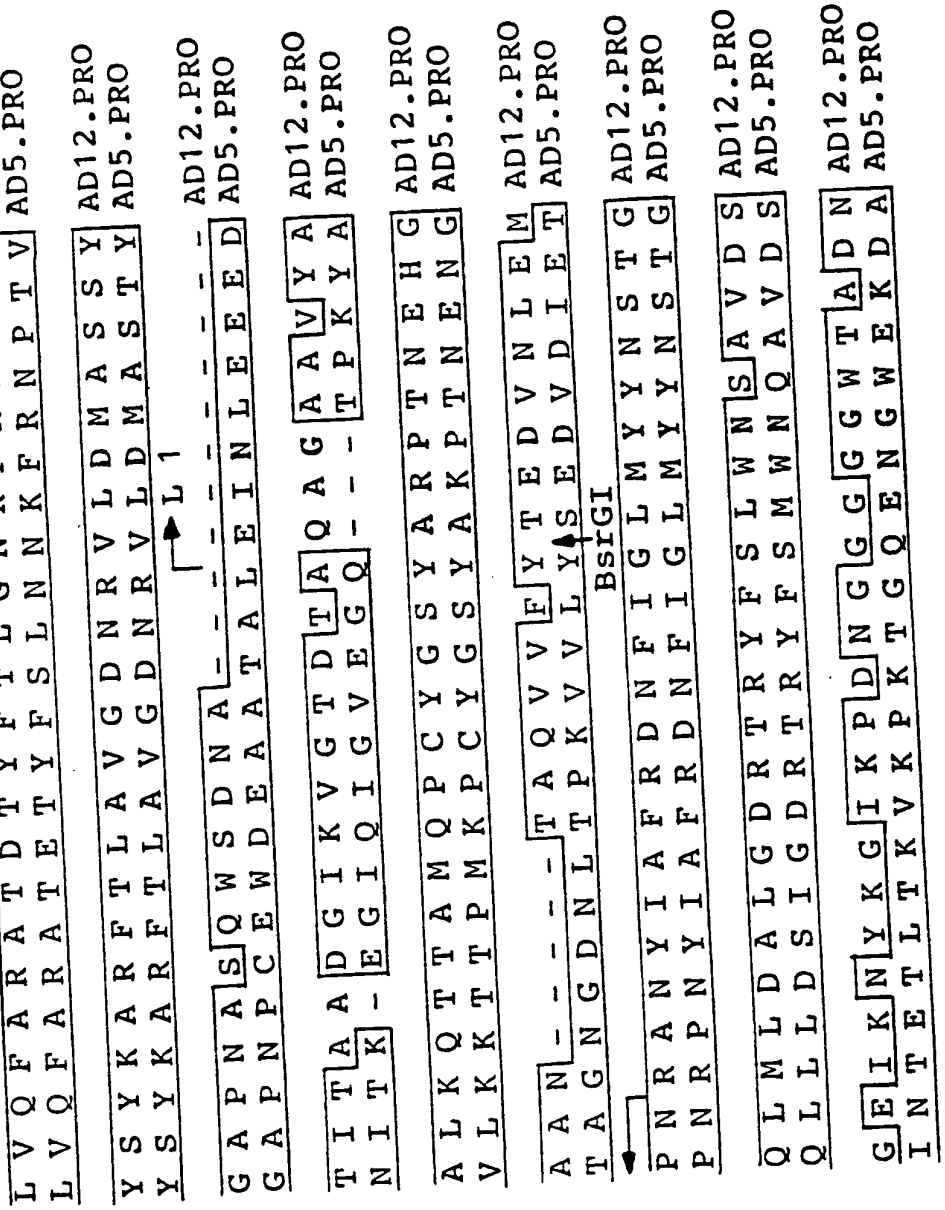
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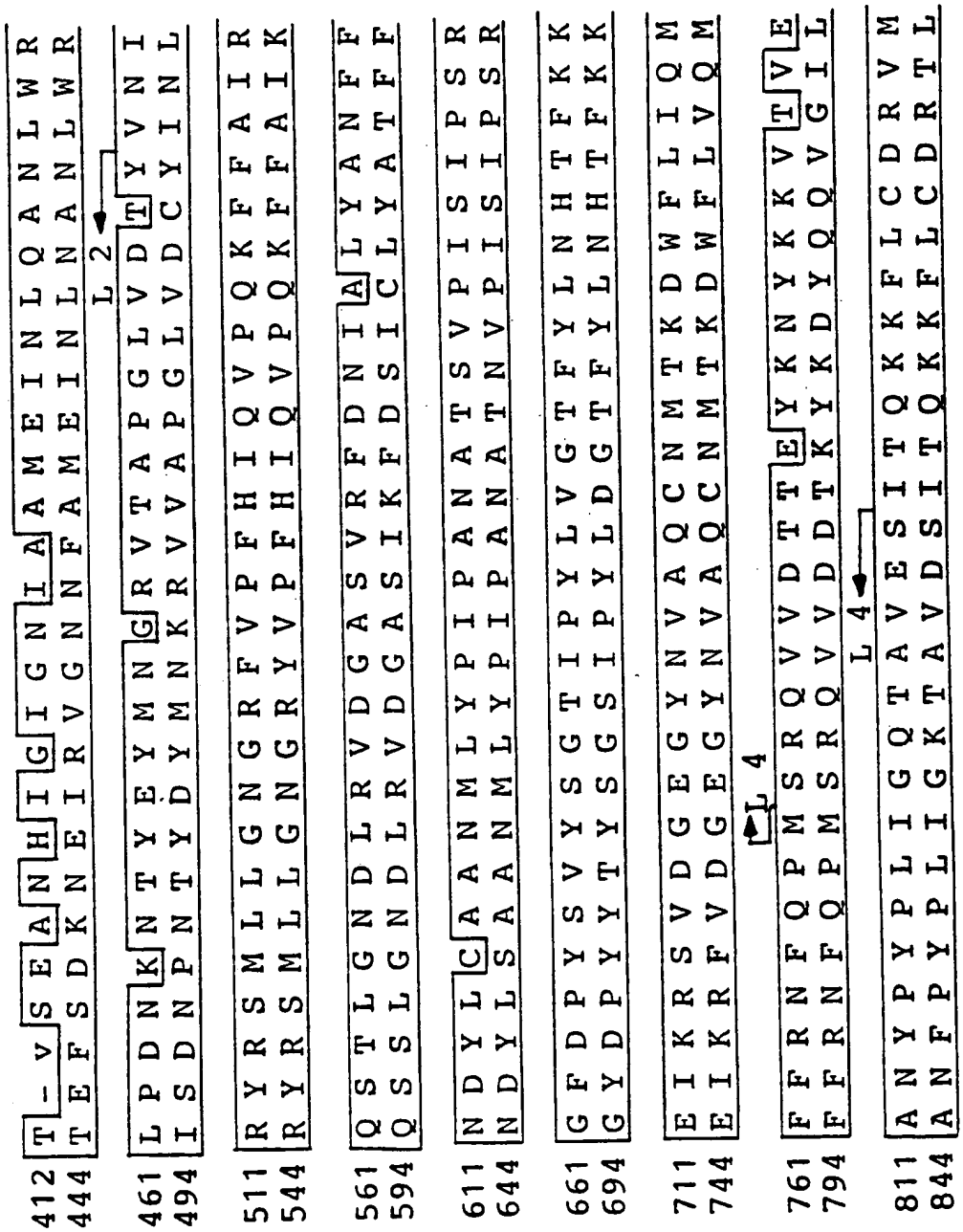
FIG. 1Ac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611 N D Y L C A A N M L Y Y P I P A N A T S V P I S I P S R
644 N D Y L S A A N M L Y Y P I P A N A T N V P I S I P S R
661 G F D P Y S V Y S G T I P Y L V G T F Y L N H T F K K
694 G Y D P Y Y T Y S G S I P Y L D G T F Y L N H T F K K
711 E I K R S V D G E G Y N V A Q C N M T K D W F L I Q M
744 E I K R F V D G E G Y N V A Q C N M T K D W F L V Q M
L 4
761 F F R N F Q P M S R Q V V D T T E Y K N Y K K V T V E
794 F F R N F Q P M S R Q V V D D T K Y K D Y Q Q V G I L
L 4
811 A N Y P Y P L I G Q T A V E S I T Q K K F L C D R V M
844 A N F P Y P L I G K T A V D S I T Q K K F L C D R T L

FIG. 1Ad

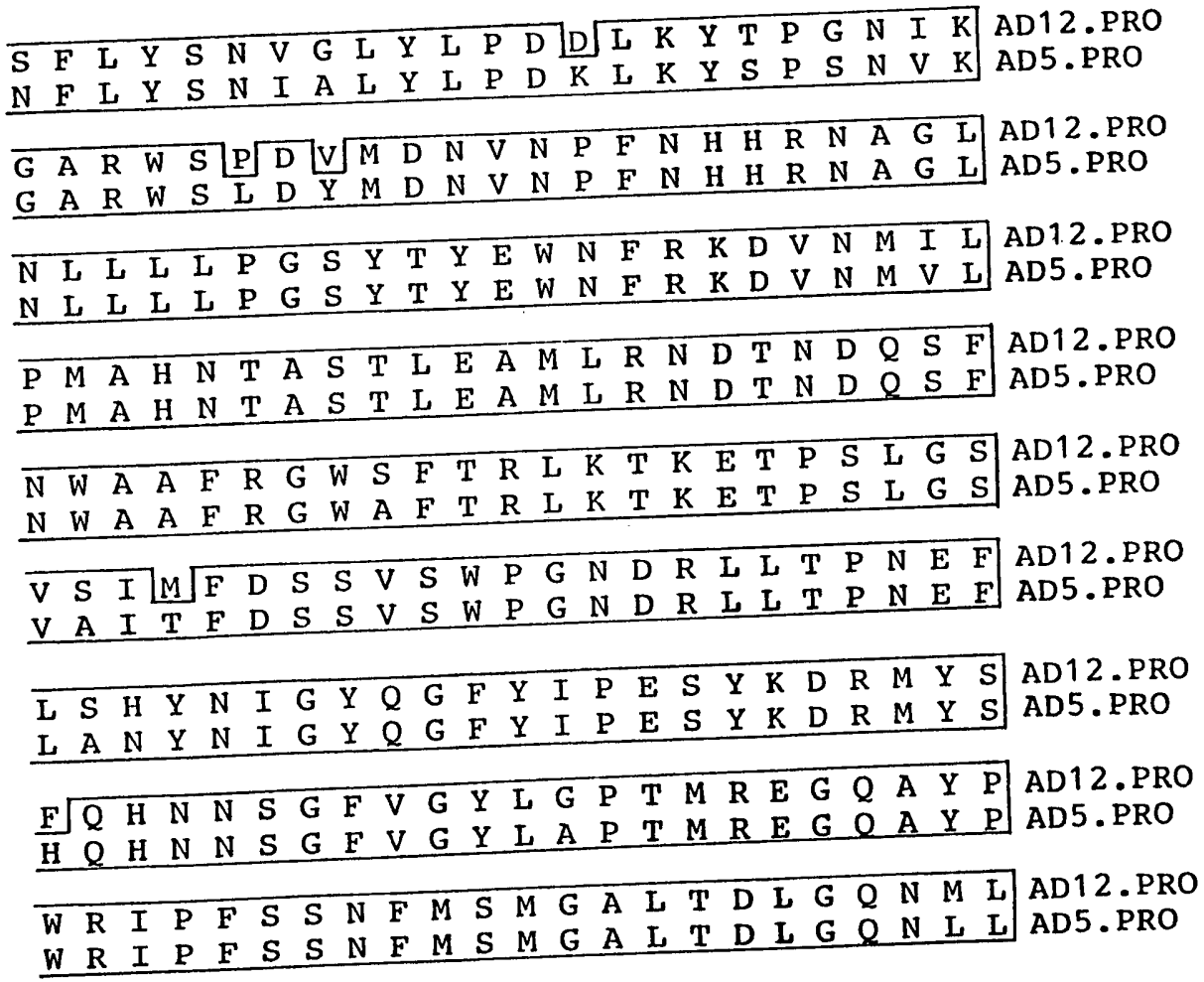

S F L Y S N V G L Y L P D D L K Y T P G N I K AD12.PRO
N F L Y S N I A L Y L P D K L K Y S P S N V K AD5.PRO
G A R W S P D V M D N V N P F N H H R N A G L AD12.PRO
G A R W S L D Y M D N V N P F N H H R N A G L AD5.PRO
N L L L L P G S Y T Y E W N F R K D V N M I L AD12.PRO
N L L L L P G S Y T Y E W N F R K D V N M V L AD5.PRO
P M A H N T A S T L E A M L R N D T N D Q S F AD12.PRO
P M A H N T A S T L E A M L R N D T N D Q S F AD5.PRO
N W A A F R G W S F T R L K T K E T P S L G S AD12.PRO
N W A A F R G W A F T R L K T K E T P S L G S AD5.PRO
V S I M F D S S V S W P G N D R L L T P N E F AD12.PRO
V A I T F D S S V S W P G N D R L L T P N E F AD5.PRO
L S H Y N I G Y Q G F Y I P E S Y K D R M Y S AD12.PRO
L A N Y N I G Y Q G F Y I P E S Y K D R M Y S AD5.PRO
F Q H N N S G F V G Y L G P T M R E G Q A Y P AD12.PRO
H Q H N N S G F V G Y L A P T M R E G Q A Y P AD5.PRO
W R I P F S S N F M S M G A L T D L G Q N M L AD12.PRO
W R I P F S S N F M S M G A L T D L G Q N L L AD5.PRO

FIG. 1Ae

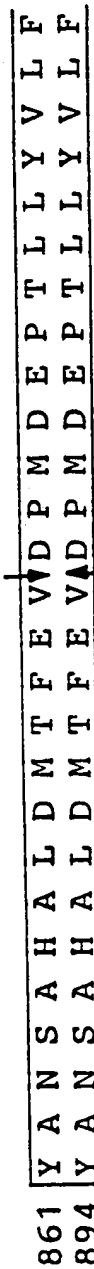


FIG. 1Ba

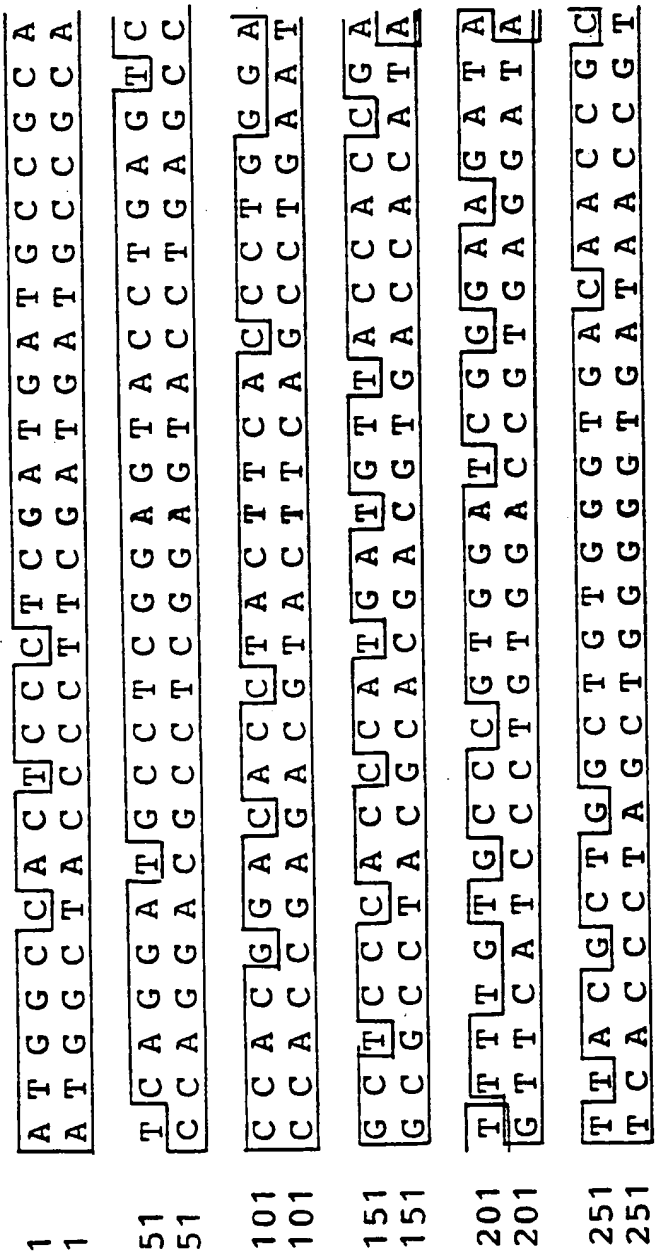


FIG. 1Af

```
E V F D V V R I H Q P H R G V I E A V Y L R T  AD12.PRO
E V F D V V R V H R P H R G V I E T V Y L R T  AD5.PRO

                                               AD12.PRO
                                               AD5.PRO
```

FIG. 1Bb

```
G T G G T C T T A C A T G C A C A T C G C C G G  AD12.SEQ
G T G G T C T T A C A T G C A C A T C T C G G G  AD5.SEQ

C C G G T C T G G T G C A A T T C G C C C G C G  AD12.SEQ
C C G G G C T G G T G C A G T T T G C C C G C G  AD5.SEQ

A A C A A G T T T A G A A A C C C C A C C G T G  AD12.SEQ
A A C A A G T T T A G A A A C C C C A C G G T G  AD5.SEQ

                      SZR46 ——>
T C G C T C G C A G C G T C T G A C G C T G C G  AD12.SEQ
C C G G T C C C A G C G T T T G A C G C T G C G  AD5.SEQ
Age1
C T A C C T A C T C C T A C A A G G C T C G C T  AD12.SEQ
C T G C G T A C T C G T A C A A G G C G C G G T  AD5.SEQ

G T G T T A G A C A T G G C T A G T T C T T A C  AD12.SEQ
G T G C T G G A C A T G G C T T C C A C G T A C  AD5.SEQ
```

FIG. 1Bc

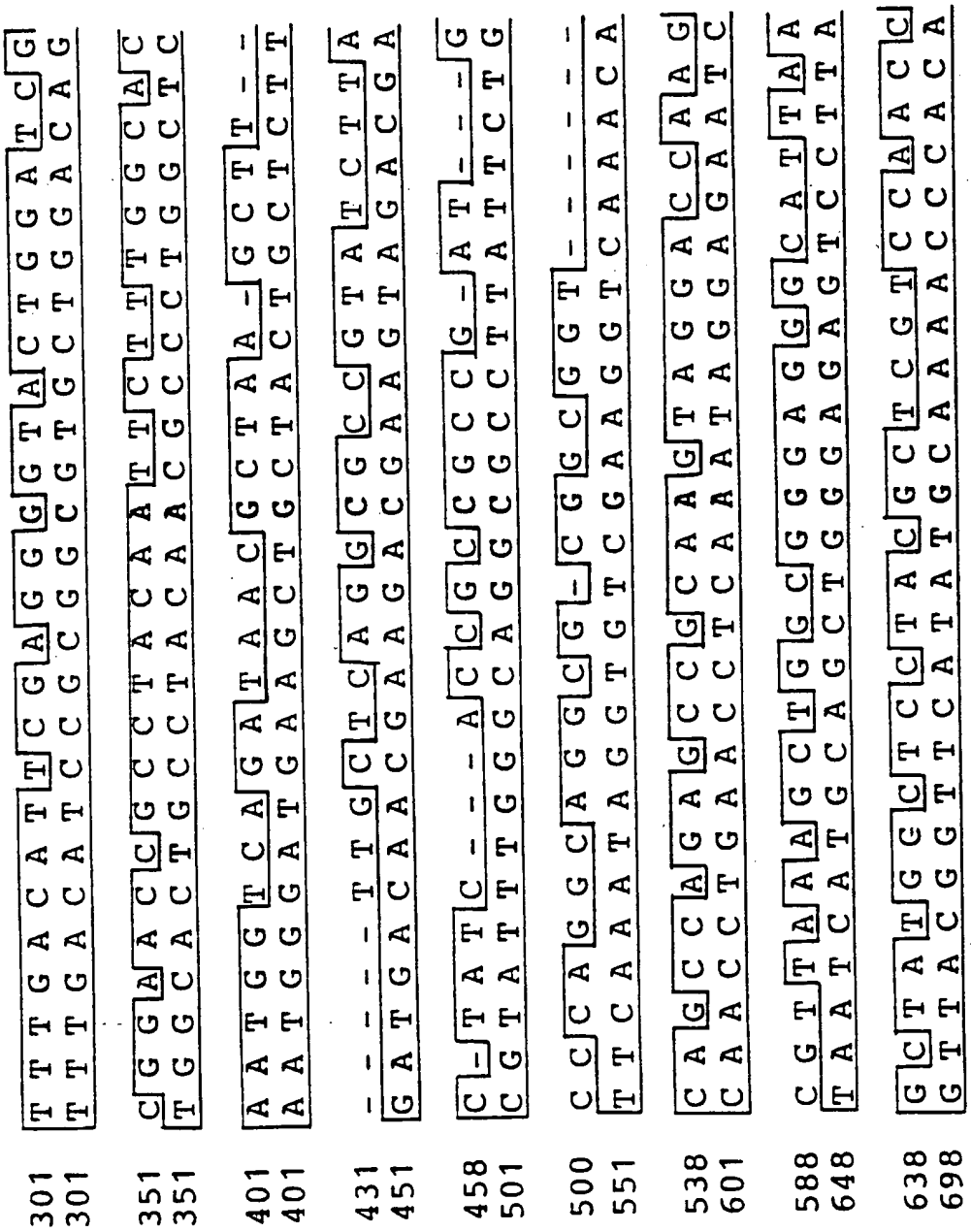

301
301
351
351
401
401
431
451
458
501
500
551
538
601
588
648
638
698

FIG. 1Bd

```
T G G T C C C A G T T T T A A G C C C T A T T C  AD12.SEQ
G G G C C C T A C T T T T A A G C C C T A C T C  AD5.SEQ

C A A A A G G C G C T C C T A A T G C T T C A C  AD12.SEQ
C C A A G G G T G C C C C A A A T C C T T G C G  AD5.SEQ

- - A A T A - - C C T - - - - - - - - - - - - -  AD12.SEQ
G A A A T A A A C C T A G A A G A A G A G G A C  AD5.SEQ

G C G A - - - - - - - - - - - - - - - - - - C A  AD12.SEQ
G C A A G C T G A G C A G C A A A A A A C T C A  AD5.SEQ

G T A T T A A A G T T G G A A C A G A C A C C G  AD12.SEQ
G T A T A A A T A T T A C A A A G G A G G G T A  AD5.SEQ

- - - - - G T A T G C C A A C A A A A C T T A T  AD12.SEQ
C C T A A A T A T G C C G A T A A A A C A T T T  AD5.SEQ

T G A A T G G A A C A C C A G C A T T G A A A A  AD12.SEQ
T C A G T G G T A - - - C G A A A C T G A A A T  AD5.SEQ

A G C A A A C C A C T G C A A T G C A G C C G T  AD12.SEQ
A A A A G A C T A C C C C A A T G A A A C C A T  AD5.SEQ

A A C G A A C A C G G A G G A C A A T C C A - -  AD12.SEQ
A A T G A A A A T G G A G G G C A A T G C A T T  AD5.SEQ
```

FIG. 1Be

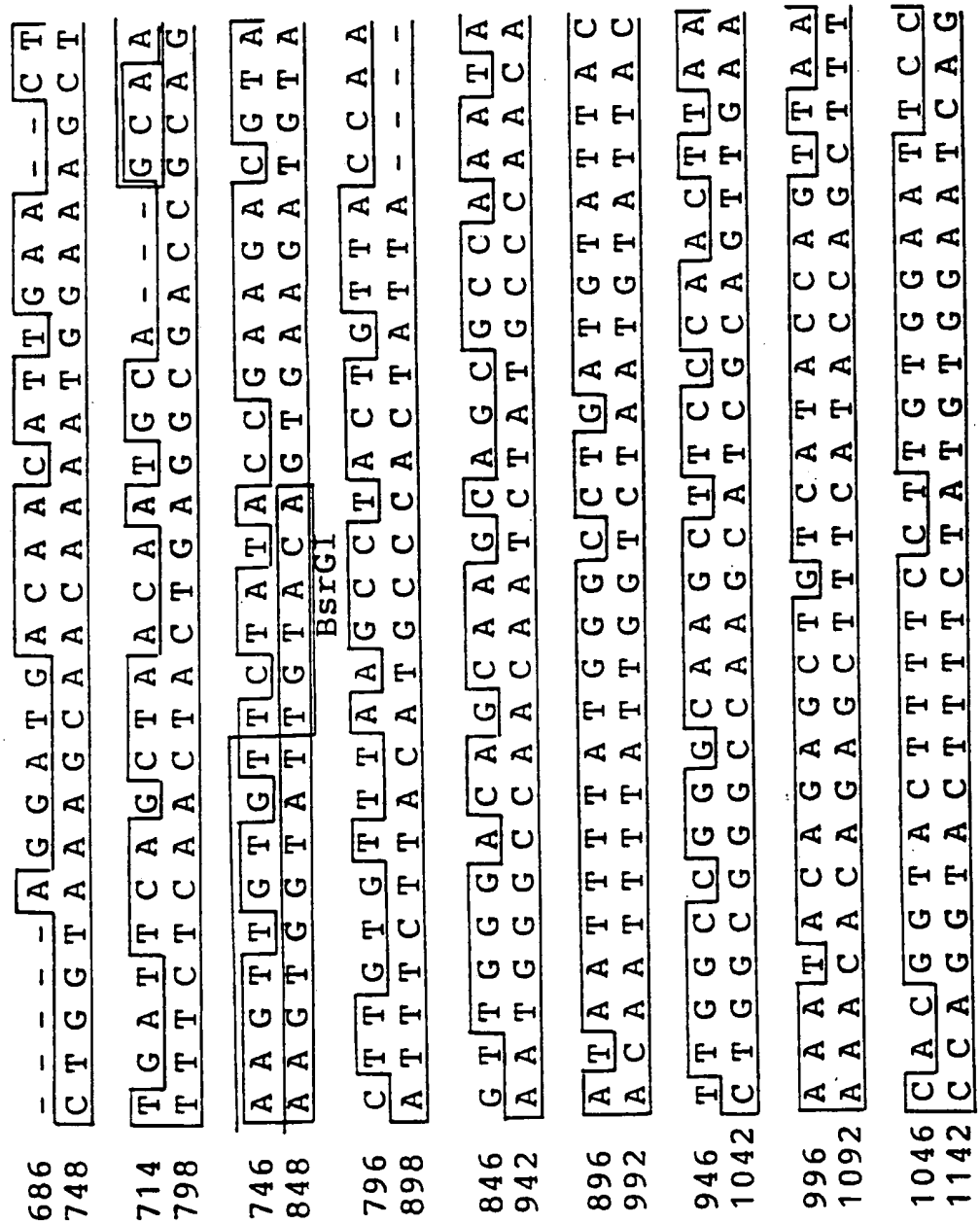

686 - - - - - A G G A T G A C A A C A T T G G A A - - C T
748 C T G G T A A A G C A A C A A A A A T G G G A A A G C T
714 T G A T T C A G C T A A C A A T G C A - - - G C A A
798 T T T C T C A A C T A C T G A G G C G A C C C G C A G
746 A A G T T G T G T C T A T A C C G A A G A C G T A
848 A A G T G G T A T T G T A C A G T G G A A G A T G T A
BsrGI
796 C T T G T G T T A A G C C T A C T G T T A C C A A
898 A T T T C T T A C A T G C C C A C T A T T A - - - -
846 G T T G G G A C A A G C A A G C G C C A A A T A
942 A A T G G G C C A A C A A T C T A T G C C C A A C A
896 A T A A T T T T A T G G G C C T G A T G T A T T A C
992 A C A A T T T T A T T G G T C T A A A T G T A T T A C
946 T T G G C C G G G C A A G C T T C C A C T T A A
1042 C T G G C G G G C C A A G C A T C G C A G T T G A A
996 A A A T A C A G A G C T G T C A T A C C A G T T A A
1092 A A A C A C A G A G C T T T C A T A C C A G C T T T
1046 C A C G G T A C T T T T C C T G T G G G A A T T C C
1142 C C A G G T A C T T T C T A T G T G G G A A T C A G

FIG. 1Bf

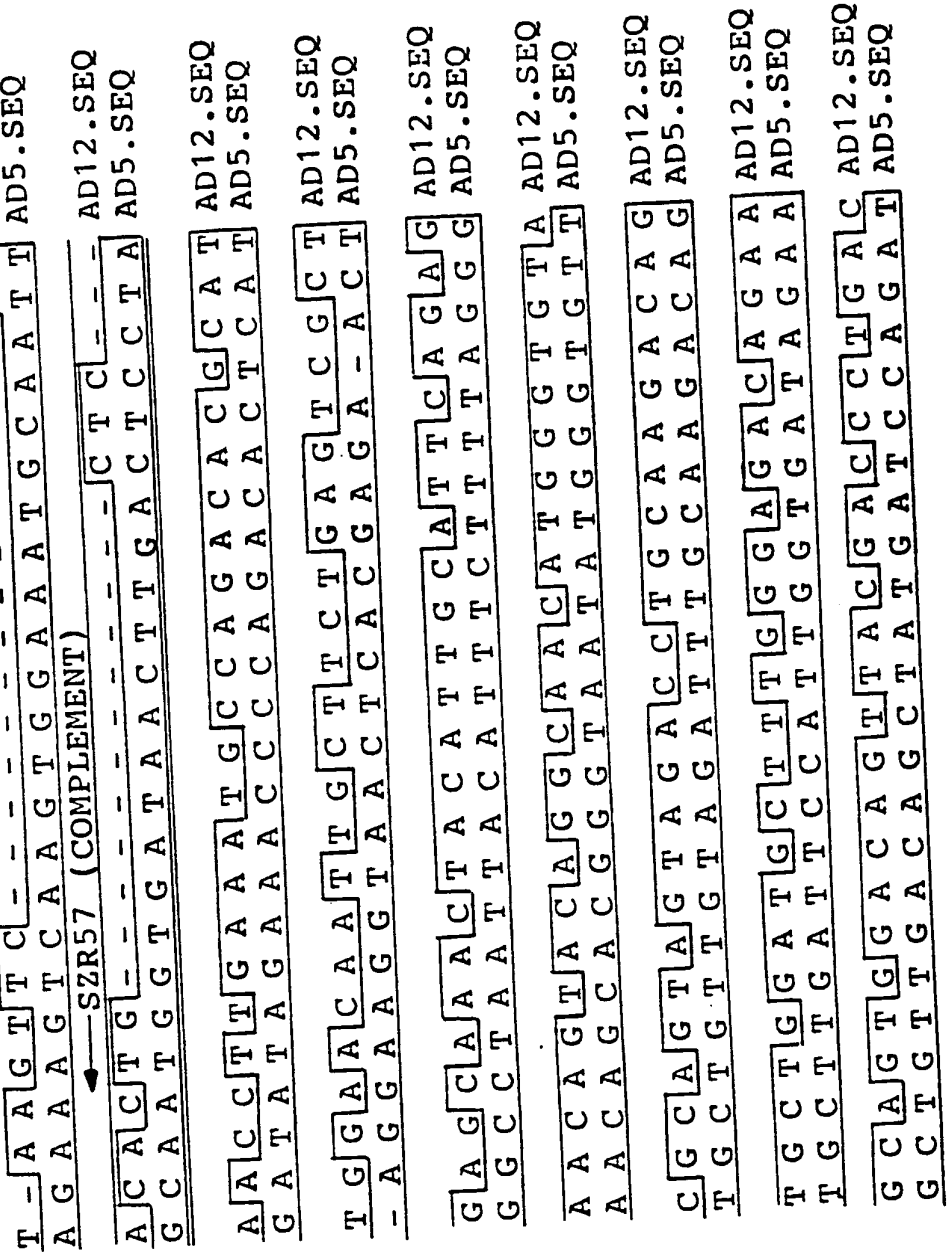

AD12.SEQ
AD5.SEQ
S2R57 (COMPLEMENT)
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ

FIG. 1B9

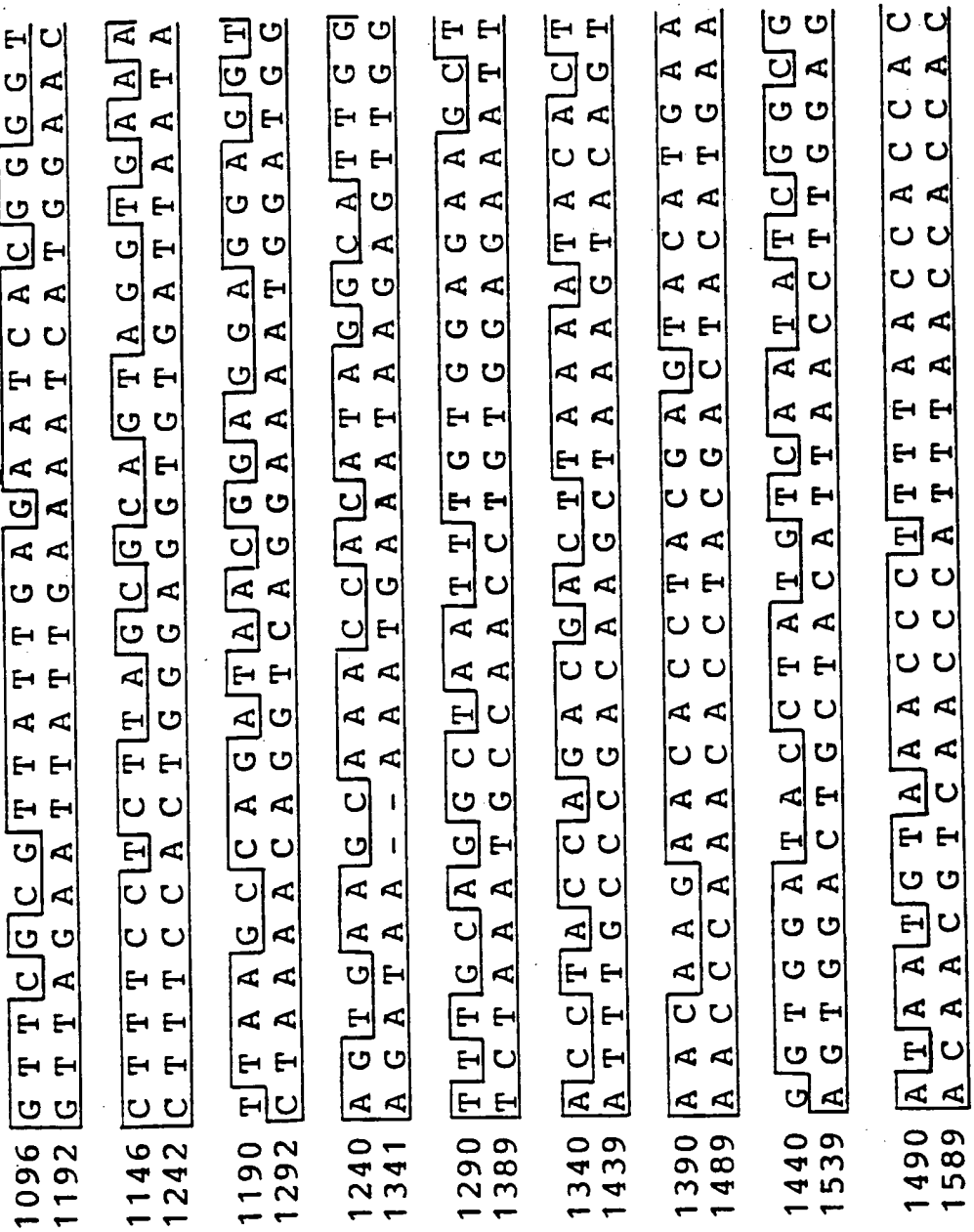

1096 GTTTCGCGTTATTGAGAATCACGGGGT
1192 GTTAGAAATTATTGAAAATTCATGGAACC
1146 CTTTTCCTCTAGCGCAGTAGGGTGAAAA
1242 CTTTCCACCTGGGAGGTGTGATTAATA
1190 TTAAAGCCAGATACGGAGGAGGGT
1292 CTAAAACAGGTCAGGAAAATGGATGG
1240 AGTGAAGCAAAACCACATAGGCATTGG
1341 AGATAA--AAATGAAATAAGAGTTGG
1290 TTTGCAGCTAATTTGTGGAGAAGCT
1389 TCTAAATGCCAACCTGTGGAAAATT
1340 ACCCTACCAGACTTAAAATACACT
1439 ATTTGCCCGACAAGCTAAAGTACAGT
1390 AACAAGAACACCTACGAGTACATGAA
1489 AACCCAAACACCTACGAACTACATGAA
1440 GGTGGATACCTATGTCAATACGGCG
1539 AGTGGACTGCTACATTAACCTTGGAG
1490 ATAATGTAAACCCCTTTAACCCACC
1589 ACAACGTCAACCCATTTAACCCACC

FIG. 1Bh

```
A G A G G A T G A A C T A C C A A A T T A T T G  AD12.SEQ
T G A A G A T G A A C T T C C A A A T T A C T G  AD5.SEQ

T A A A A A A T - - T A C A A A G G C A - - - -  AD12.SEQ
C A G A G A C T C T T A C C A A G G T A A A A C  AD5.SEQ

G G C T G G A C T G C C G A C A A C A C T G T C  AD12.SEQ
G A A A A A G A T G C T - A C A G A A T T T T C  AD5.SEQ

G A A T A T A G C C G C C A T G G A A A T T A A  AD12.SEQ
A A A T A A T T T T G C C A T G G A A A T C A A  AD5.SEQ

T C T T G T A C T C A A A T G T G G G C T T A T  AD12.SEQ
T C C T G T A C T C C A A C A T A G C G C T G T  AD5.SEQ

C C A G G A A A C A T A A A A C T A C C T G A T  AD12.SEQ
C C T T C C A A C G T A A A A A T T T C T G A T  AD5.SEQ

C G G G C G T G T G A C T G C C C C G G G G T T  AD12.SEQ
C A A G C G A G T G G T G G C T C C C G G G T T  AD5.SEQ

C T C G C T G G T C C C C A G A T G T G A T G G  AD12.SEQ
C A C G C T G G T C C C T T G A C T A T A T G G  AD5.SEQ

C G A A A C G C A G G G T T G C G C T A C A G A  AD12.SEQ
C G C A A T G C T G G C C T G C G C T A C C G C  AD5.SEQ
```

FIG. 1Bj

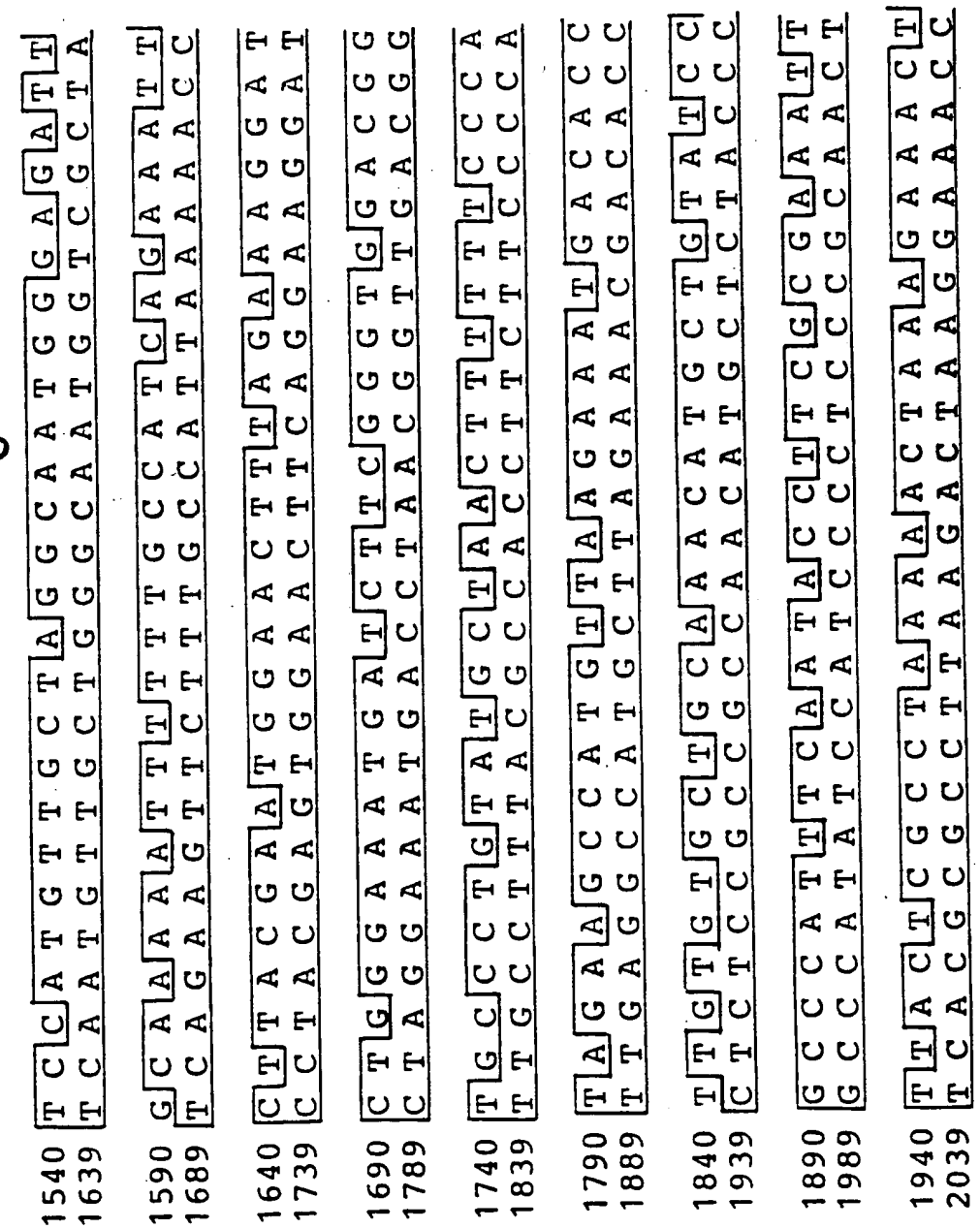

1540 TCCATTGTTGCTAGGCAAATGGGAGGATT
1639 TCAATGTTGCTGGGGCAATGGTCGCTA
1590 GCAAAATTTTTGCCATCAGAAATT
1689 TCAGAAGTTCTTTGCCATTAAAAACC
1640 CTTACGAATGGAACTTTAGAAGGAT
1739 CCTACGAGTGGAACTTCAGGGAAGAT
1690 CTGGGAAATGATCTTCGGGTGGACGG
1789 CTAGGAAATGACCTAACGGGTTGACGG
1740 TGCCCTGTAATGCTAACTTTTCCCA
1839 TTGCCTTTACGCCACCTTCTCCCCA
1790 TAGAAGCCATGTTAGAAATGACCAC
1889 TTGAGGCCATTGCTTAGAAACGACC
1840 TTGTGTGCTGCCAACATGCCTGTACC
1939 CTCTCCGCCGCCAACATGCCTCTACC
1890 GCCCCATTTCATACCCTCGCGAATT
1989 GCCCCATATCCCATCCCCCGCAACT
1940 TTACTCGCCCTAAAAACTAAGAAACT
2039 TCACGCGCCTTAAGACTAAGGAAACC

FIG. 1BK

```
T G T T C C T T T T C A C A T T C A G G T G C C  AD12.SEQ
T G T G C C C T T C C A C A T C C A G G T G C C  AD5.SEQ

T G T T G C T G T T G C C C G G T T C C T A C A  AD12.SEQ
T C C T T C T C C T G C C G G G C T C A T A C A  AD5.SEQ

G T A A A C A T G A T T C T T C A G A G C A C A  AD12.SEQ
G T T A A C A T G G T T C T G C A G A G C T C C  AD5.SEQ

A G C C A G C G T T C G C T T T G A C A A C A T  AD12.SEQ
A G C C A G C A T T A A G T T T G A T A G C A T  AD5.SEQ

T G G C A C A T A A C A C A G C T T C T A C T T  AD12.SEQ
T G G C C C A C A A C A C C G C C T C C A C G C  AD5.SEQ

A A C G A C C A G T C T T T T A A C G A T T A T  AD12.SEQ
A A C G A C C A G T C C T T T A A C G A C T A T  AD5.SEQ

C A T C C C A G C T A A C G C C A C C A G C G T  AD12.SEQ
T A T A C C C G C C A A C G C T A C C A A C G T  AD5.SEQ

G G G C G G C A T T T A G A G G C T G G A G C T  AD12.SEQ
G G G C G G C T T T C C G C G G C T G G G C C T  AD5.ASQ

C C T T C C C T G G G T T C A G G G T T T G A C  AD12.SEQ
C C A T C A C T G G G C T C G G G C T A C G A C  AD5.SEQ
```

FIG. 1B1

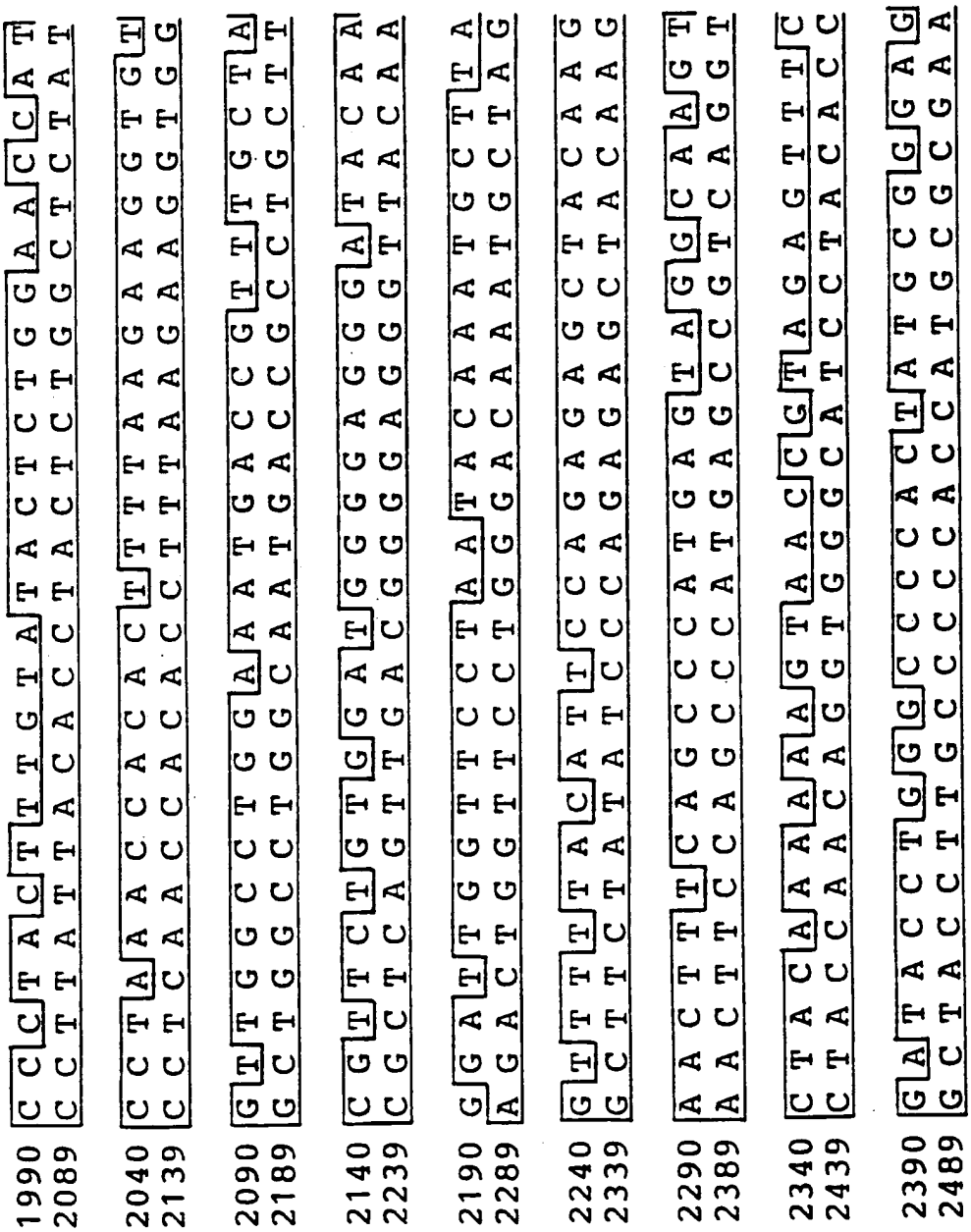

1990 CCCTACTTGGTACTTCTGGAACCAT
2089 CCTTATTACCACCTACCTCTGGCTCCTAT
2040 CCTTAAACCACACTTTTAAGAAGGGTGT
2139 CCTCAACCACCTTTAAGAAGGTGG
2090 GTTGGCCTGGAATGGACCGTTTGCTA
2189 GCTGGCCTGGCAATGACCGCCTGCTT
2140 CGTTCTGTGGATGGGGGAGGGATACAA
2239 CGCTCAGTTGACGGGGAGGGTTACAA
2190 GGATTGGTTCCTAATACAAATGCTTA
2289 AGACTGGTTCCTGGGACAAATGCTAG
2240 GTTTTACATTCCAGAGAGCTACAAG
2339 GCTTCTATTCCCAGAGCTACAAG
2290 AACTTTCAGCCCCATGAGTAGGCAGT
2389 AACTTCCAGCCCCATGAGCCGTCAGGT
2340 CTACAAAAAGTAACGTAGAGTTTC
2439 CTACCAACAGGTGGGCATCCTACACC
2390 GATACCTGGGCCCCACTATGCGGAG
2489 GCTACCTTGCCCCCACCATGCCGAA

FIG. 1Bm

```
T C C C T A T T T A G A C G G C A C C T T T T A   AD12.SEQ
A C C C T A C C T A G A T G G A A C C T T T T A   AD5.SEQ

C A A T C A T G T T T G A C T C C T C C G T G A   AD12.SEQ
C C A T T A C C T T T G A C T C T T C T G T C A   AD5.SEQ

A C C C C A A A T G A A T T T G A A A T A A A G   AD12.SEQ
A C C C C C A A C G A G T T T G A A A T T A A G   AD5.SEQ

T G T G G C C C A A T G C A A T A T G A C T A A   AD12.SEQ
C G T T G C C C A G T G T A A C A T G A C C A A   AD5.SEQ

G T C A T T A C A A C A T T G G A T A C C A A G   AD12.SEQ
C T A A C T A C A A C A T T G G C T A C C A G G   AD5.SEQ

G A C C G C A T G T A T T C T T T C T T T A G A   AD12.SEQ
G A C C G C A T G T A C T C C T T C T T T A G A   AD5.SEQ

T G T G G A T A C C A C A G A A T A T A A G A A   AD12.SEQ
G G T G G A T G A T A C T A A A T A C A A G G A   AD5.SEQ

A A C A T A A C A A C T C A G G A T T C G T G G   AD12.SEQ
A A C A C A A C A A C T C T G G A T T T G T T G   AD5.SEQ

G G A C A A G C T T A C C C C G C C A A C T A T   AD12.SEQ
G G A C A G G C C T A C C C T G C T A A C T T C   AD5.SEQ
```

FIG. 1Bn

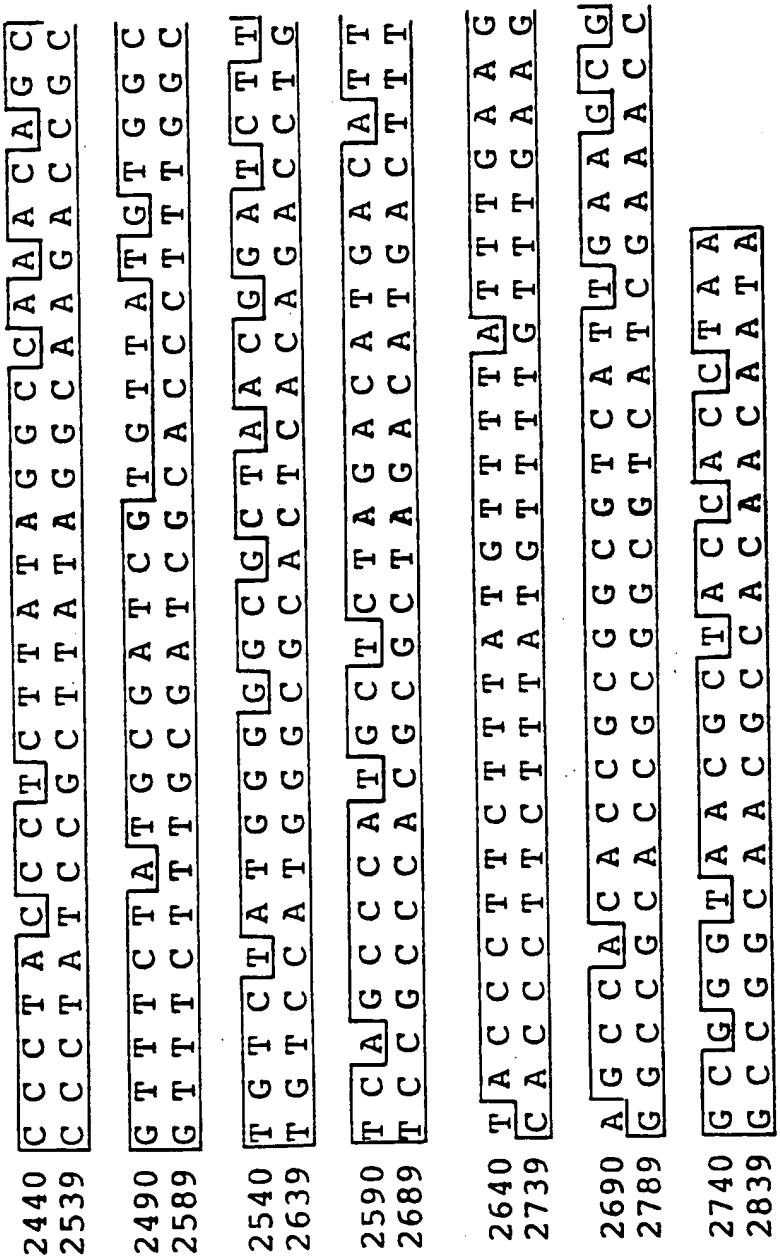

2440 CCCCTACCCTCTTATAGGGCCAACAGCC
2539 CCCTATCCGCTTATAGGCCAAGACCGCC
2490 GTTTTCCTATCGCGATCGTGTTATGTGGC
2589 GTTTTCCTTTGCGCGATCGCCACCCCTTTGGCC
2540 TGTTCTATGGGGGCGCTAACGGATCTCT
2639 TGTCCCATGGGGCGCCTCACCAGGACCCTG
2590 TCAGCCCCATGCTCTAGACCATGACATTT
2689 TCCGCCCCACGCGCTAGACCATGACCTTT
2640 TACCCCTTCTTTTATGTTTTTATTTTGAAAG
2739 CACCCTTCTTTATGTTTTTGTTTTGAAAG
2690 AGCCCACCCCGCCGTCATTGAAAGCGG
2789 GGCCCGCACCCGCGGCGTCATCGAAACCC
2740 GCGGGGTACACGCTACCCTAA
2839 GCCGGGCAACGCCCAACCAATA

FIG. 1Bo

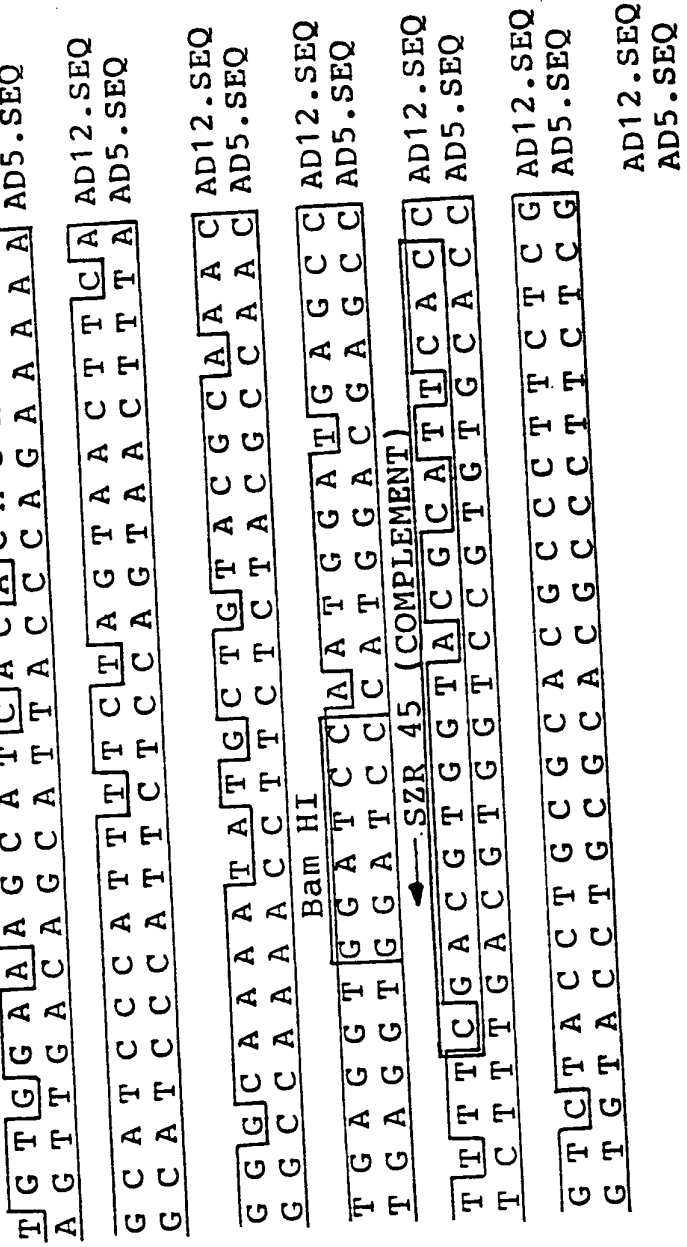

AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
Bam HI
AD12.SEQ
AD5.SEQ
SZR 45 (COMPLEMENT)
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ
AD12.SEQ
AD5.SEQ

FIG. 2

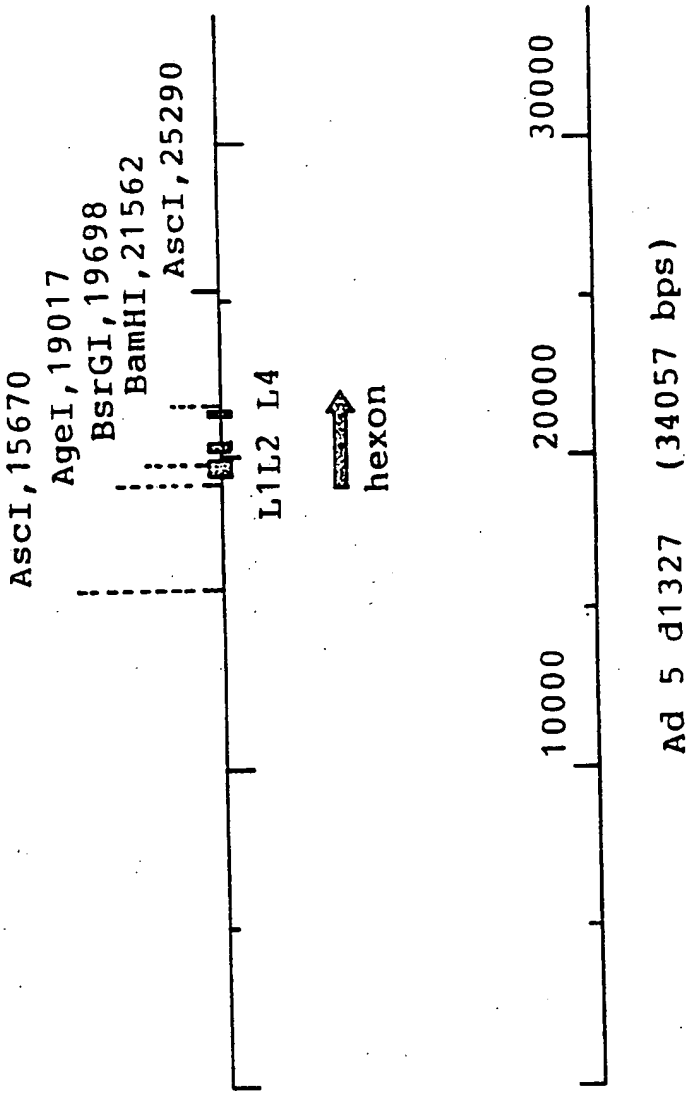

AscI,15670
AgeI,19017
BsrGI,19698
BamHI,21562
AscI,25290
L1 L2 L4
hexon
10000
20000
30000
Ad 5 d1327 (34057 bps)

FIG. 3A

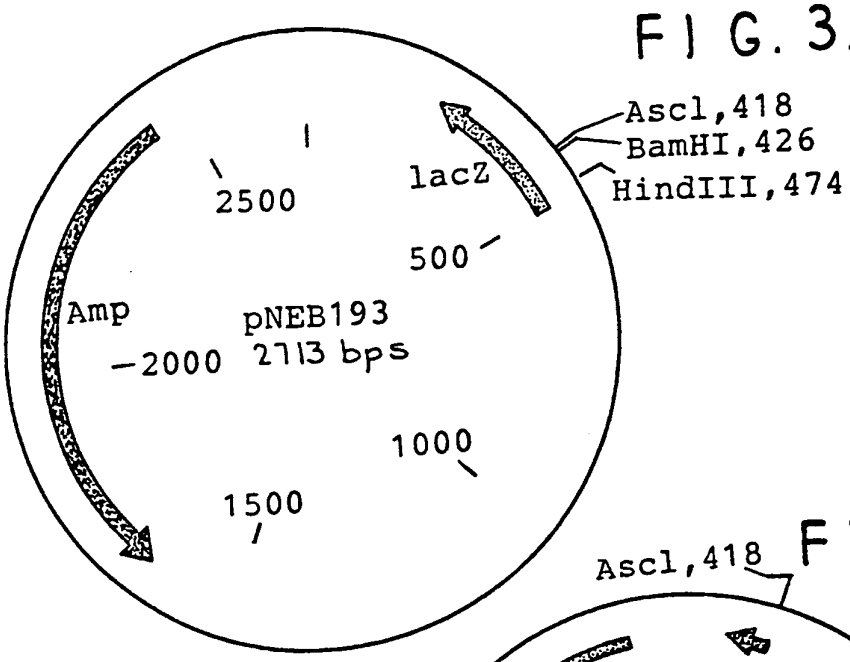
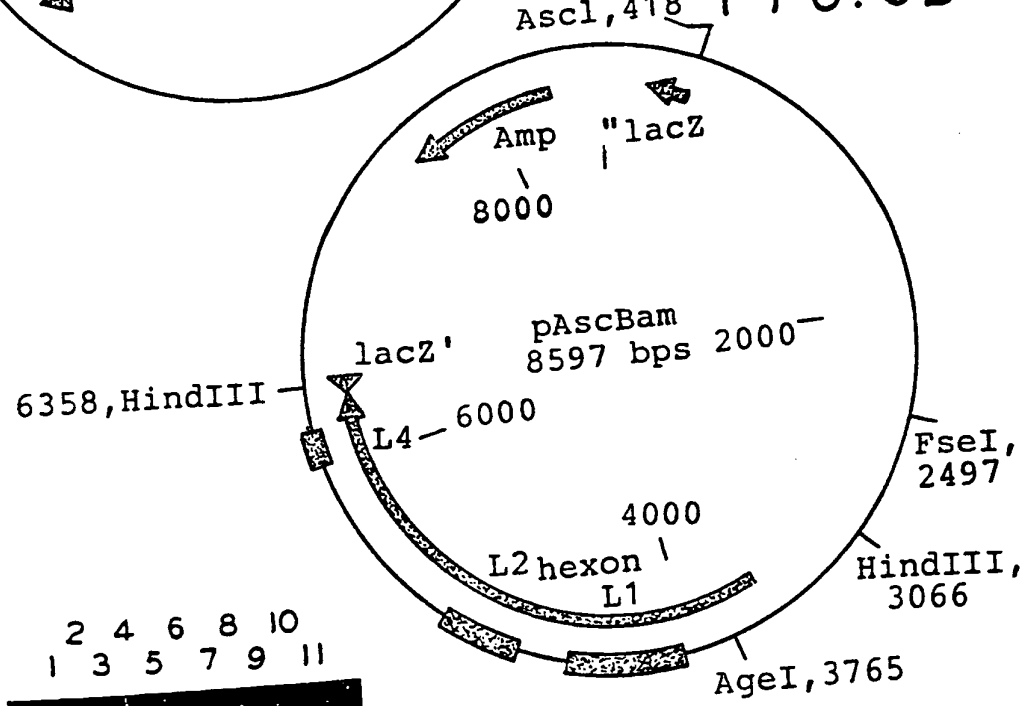


FIG. 3B



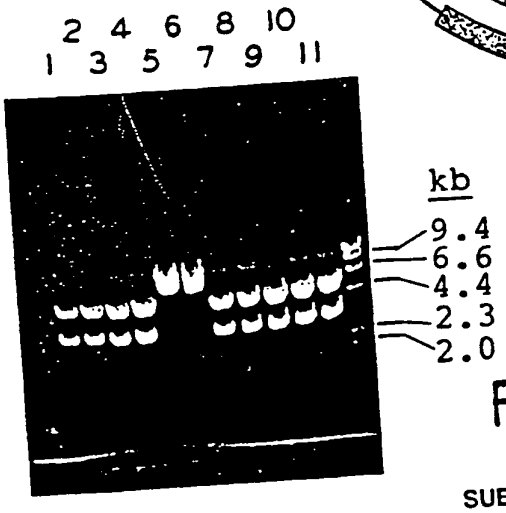


FIG. 3C

FIG. 4A

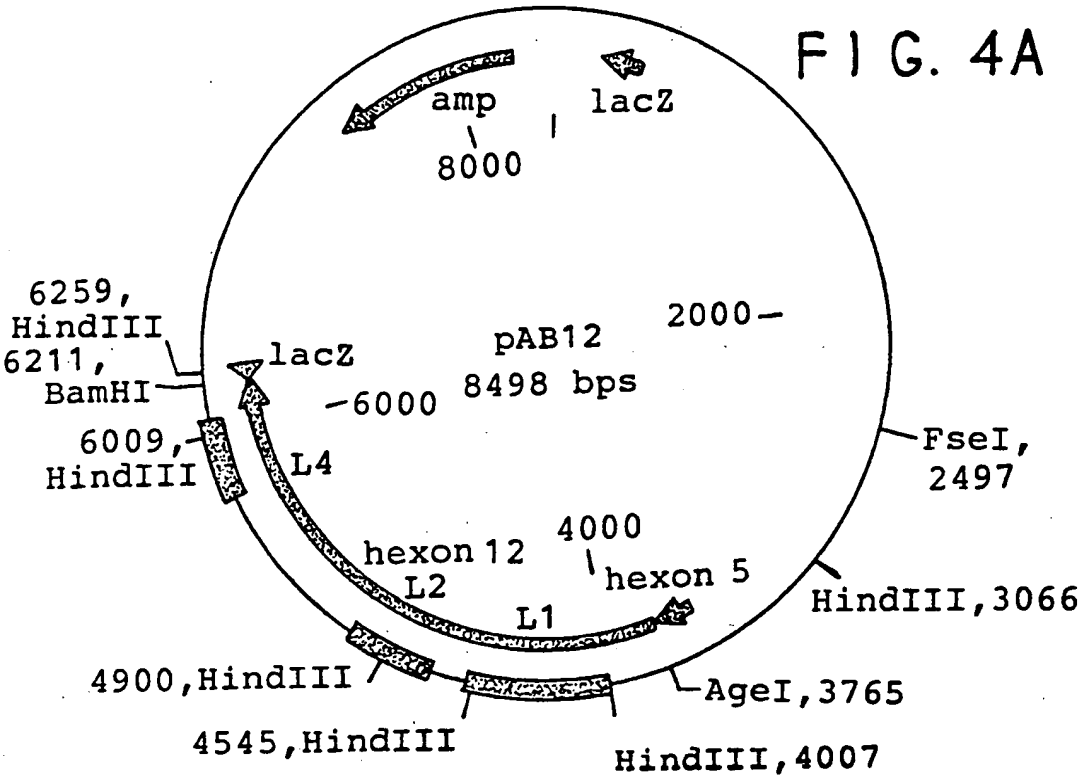

amp
lacZ
8000
6259,
HindIII
6211,
BamHI
lacZ
pAB12
8498 bps
2000
6000
6009,
HindIII
L4
FseI,
2497
4000
hexon 12
L2
hexon 5
L1
HindIII,3066
4900,HindIII
AgeI,3765
4545,HindIII
HindIII,4007

FIG. 4B

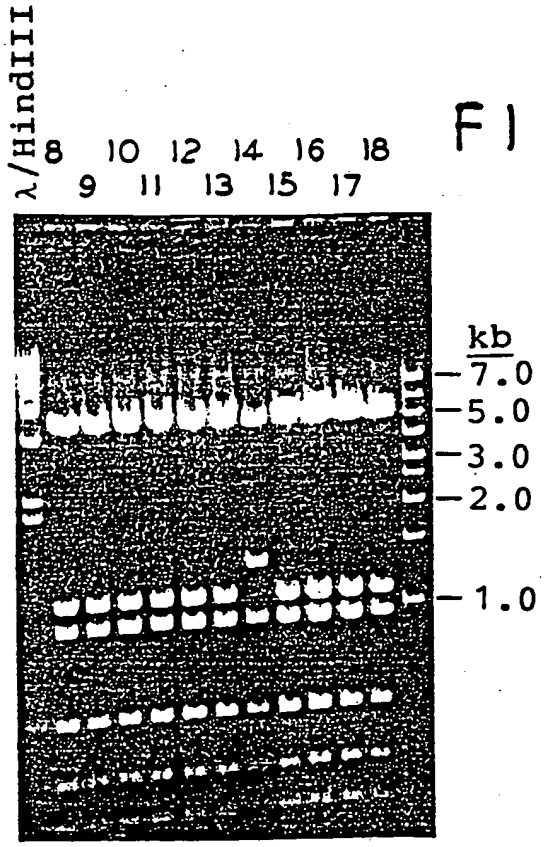

λ/HindIII
8 10 12 14 16 18
9 11 13 15 17
kb
7.0
5.0
3.0
2.0
1.0

FIG. 4C

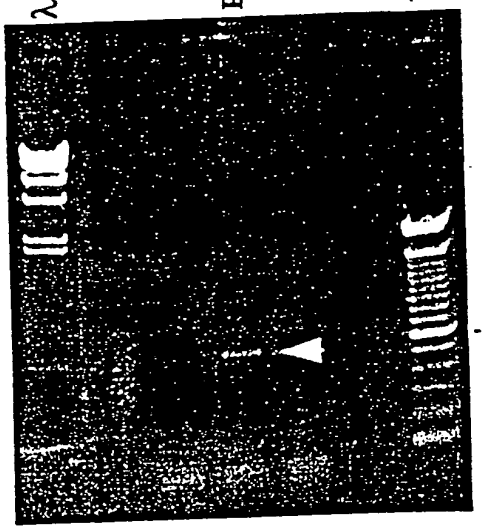


FIG. 4D

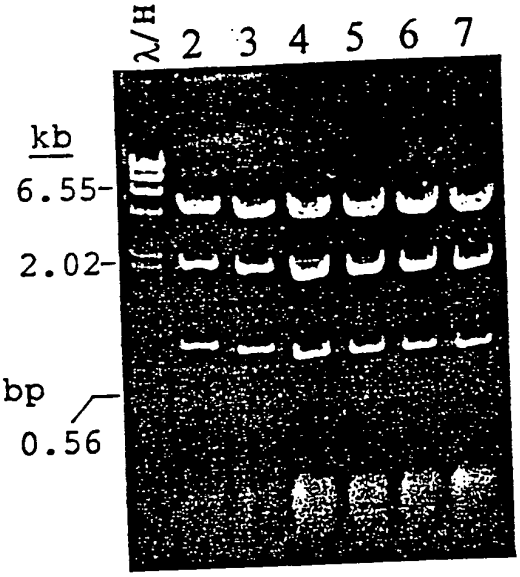


FIG. 4E

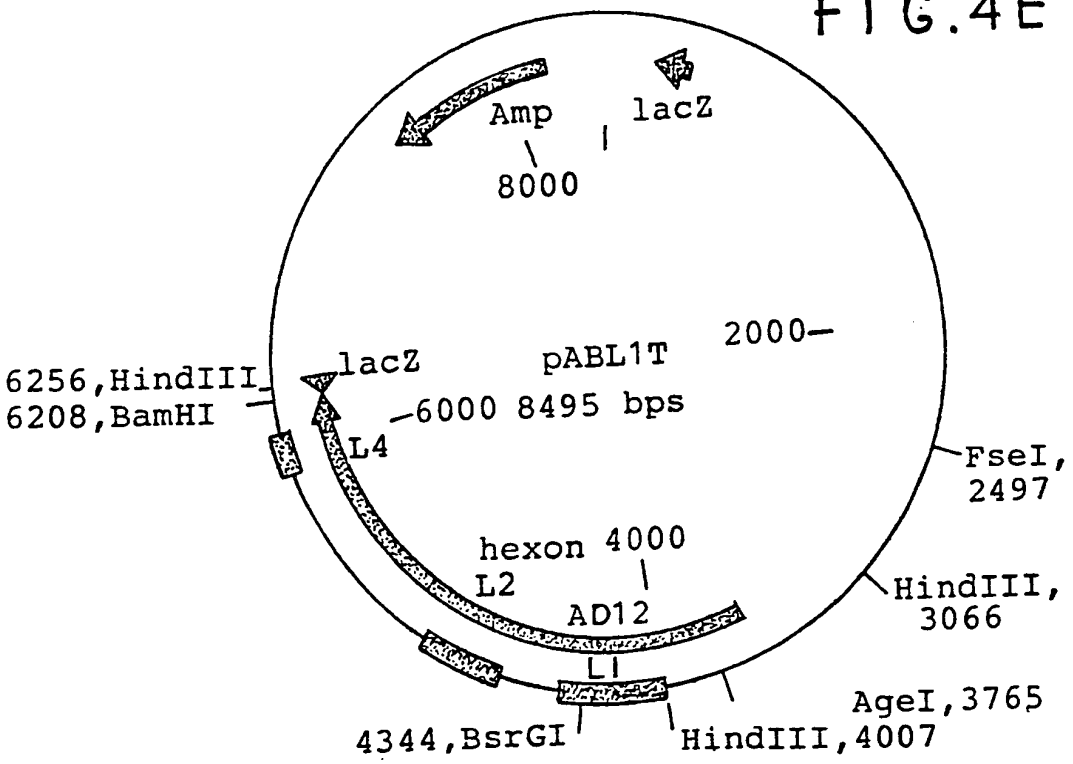


FIG.5A

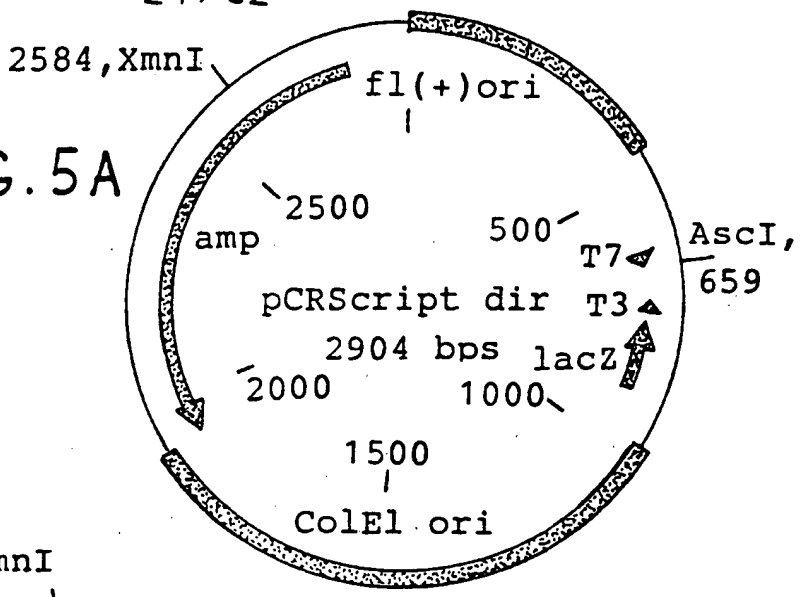


FIG.5B

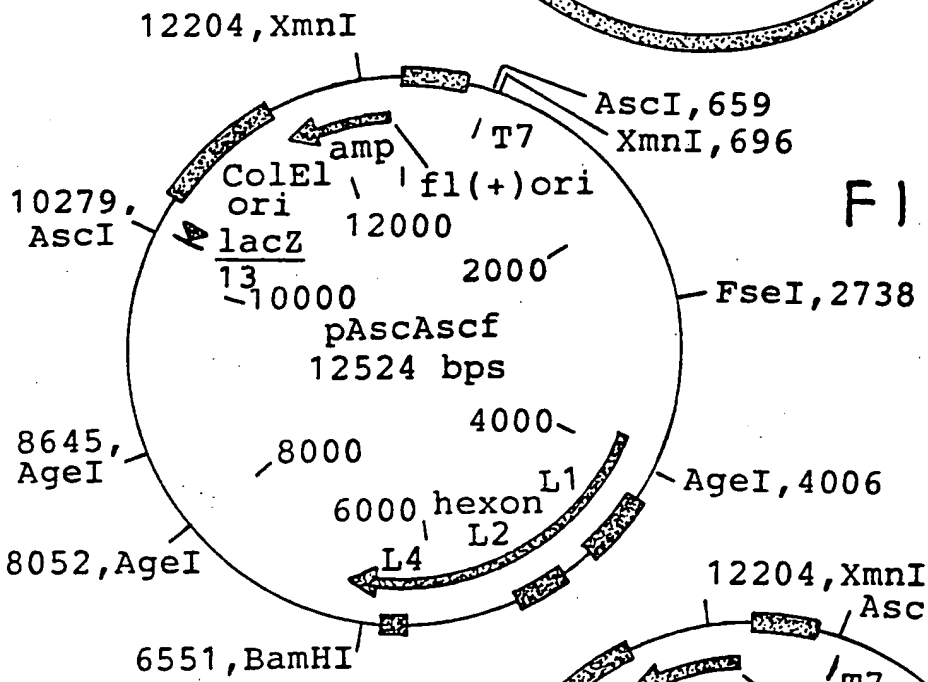


FIG.5C

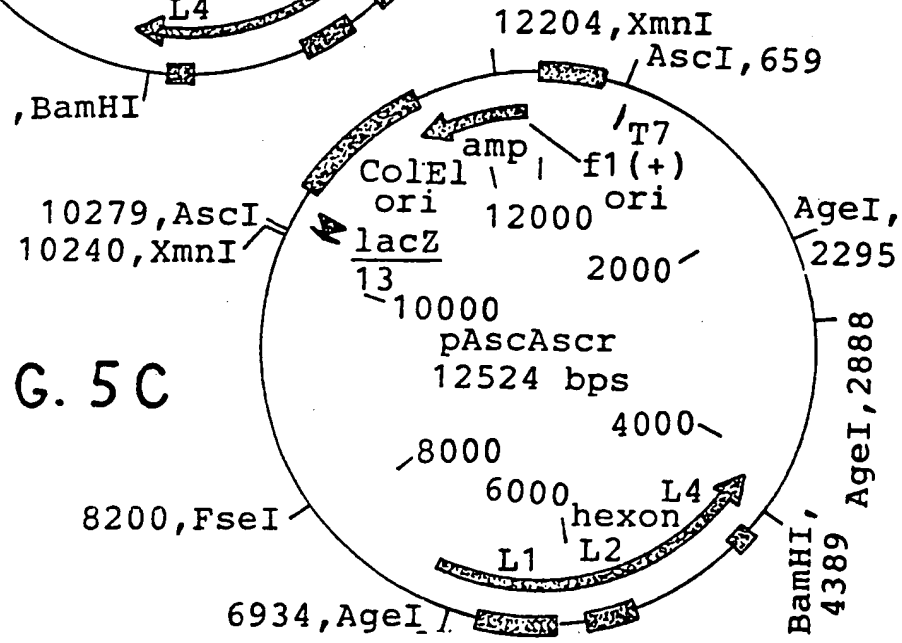


FIG. 5D

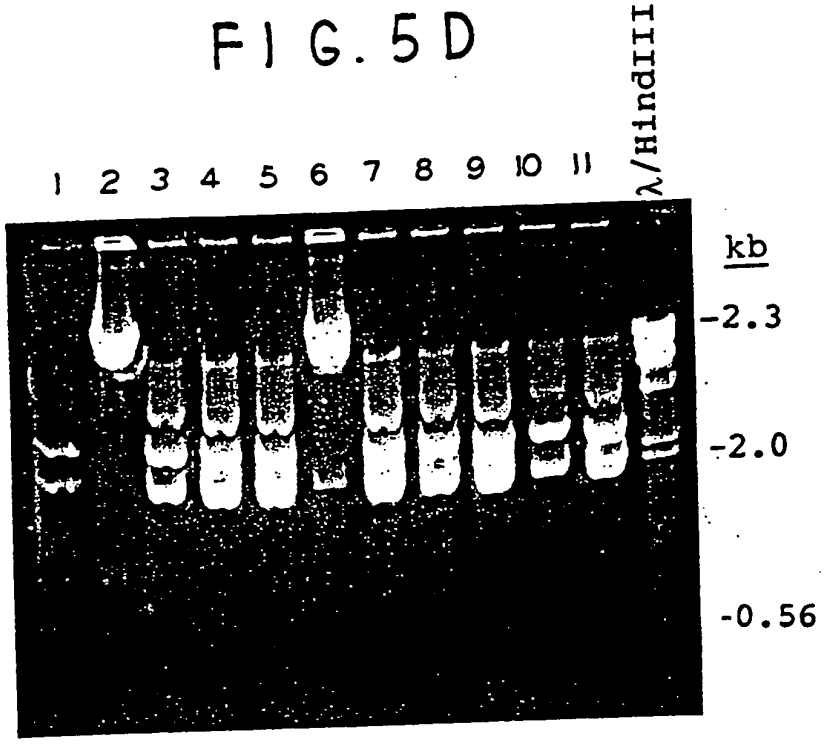

1 2 3 4 5 6 7 8 9 10 11
λ/HindIII
kb
-2.3
-2.0
-0.56

FIG. 5E

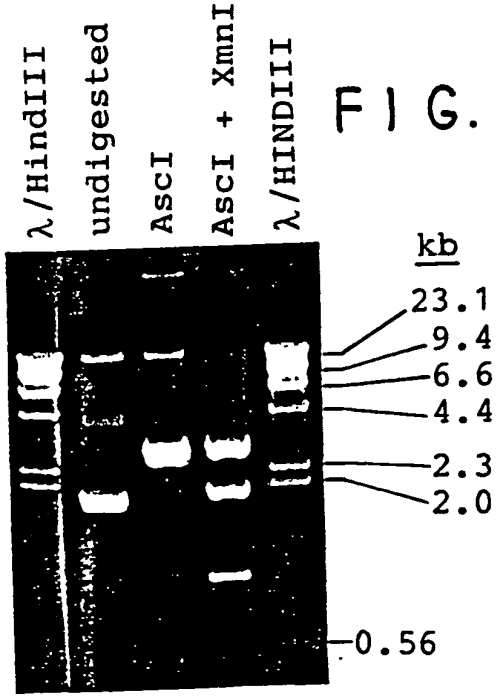

λ/HindIII
undigested
AscI
AscI + XmnI
λ/HINDIII
kb
23.1
9.4
6.6
4.4
2.3
2.0
0.56

FIG. 6A

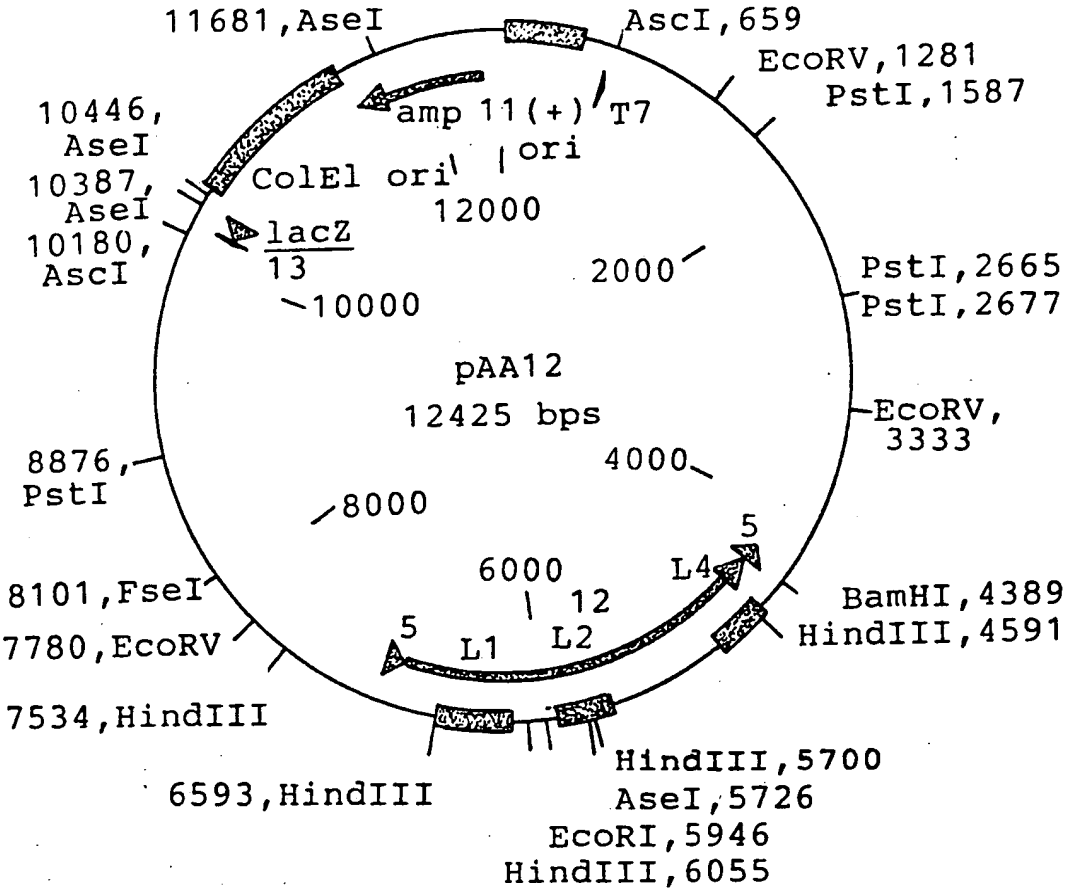

11681,AseI
AscI,659
EcoRV,1281
PstI,1587
10446,
AseI
amp
11(+)
T7
ori
10387,
AseI
ColEl ori
12000
10180,
AscI
lacZ
13
2000
PstI,2665
PstI,2677
10000
pAA12
12425 bps
EcoRV,
3333
8876,
PstI
4000
8000
5
6000
L4
12
8101,FseI
5
L1
L2
BamHI,4389
HindIII,4591
7780,EcoRV
7534,HindIII
HindIII,5700
AseI,5726
6593,HindIII
EcoRI,5946
HindIII,6055

FIG. 6B

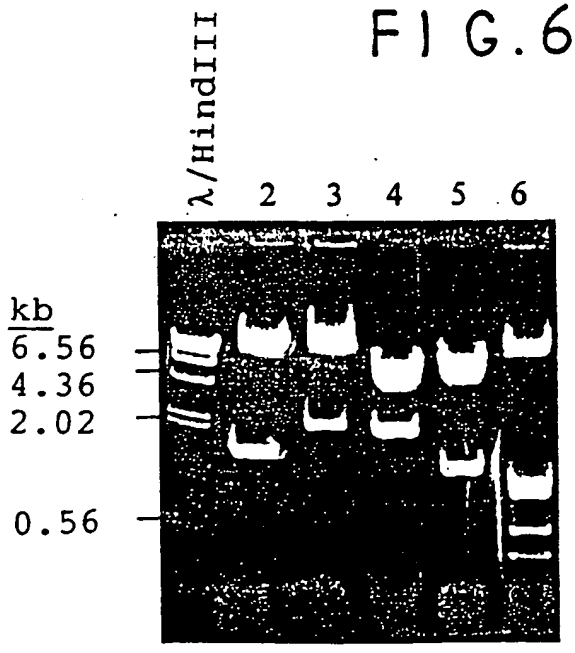

λ/HindIII
2
3
4
5
6
kb
6.56
4.36
2.02
0.56

FIG. 7A

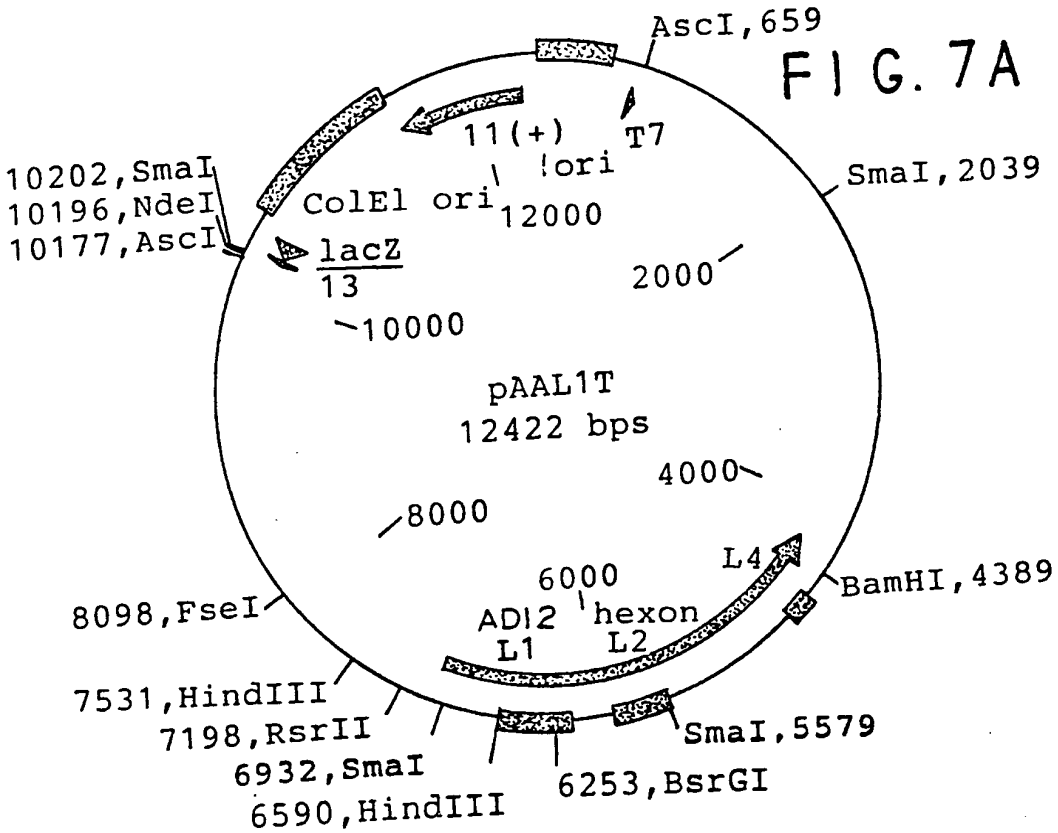


FIG. 7B

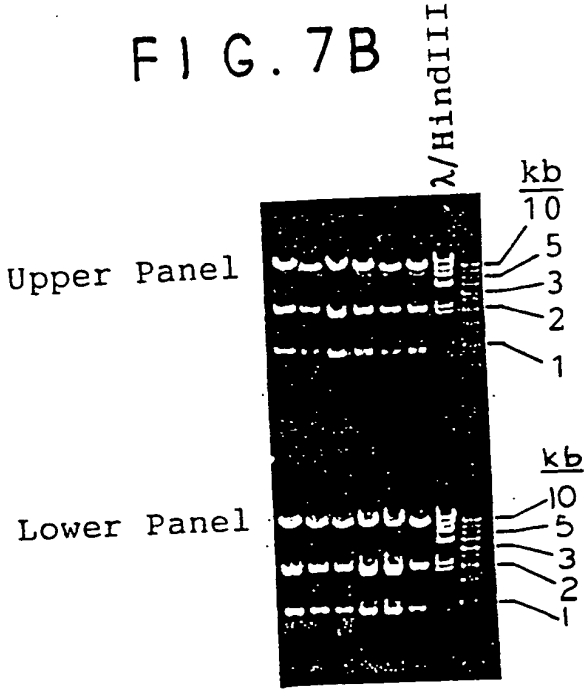


FIG. 7C

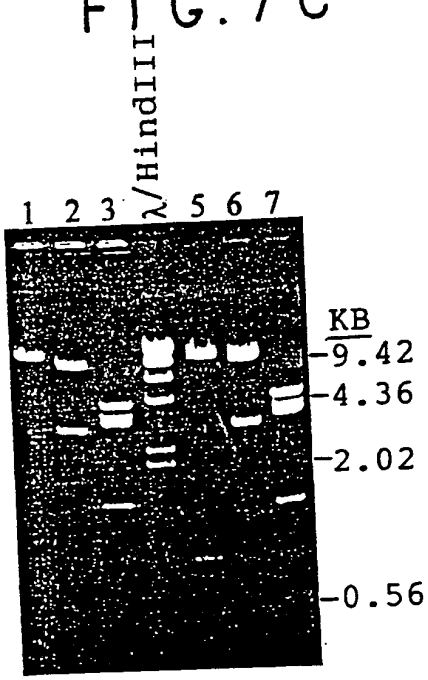


FIG. 8A

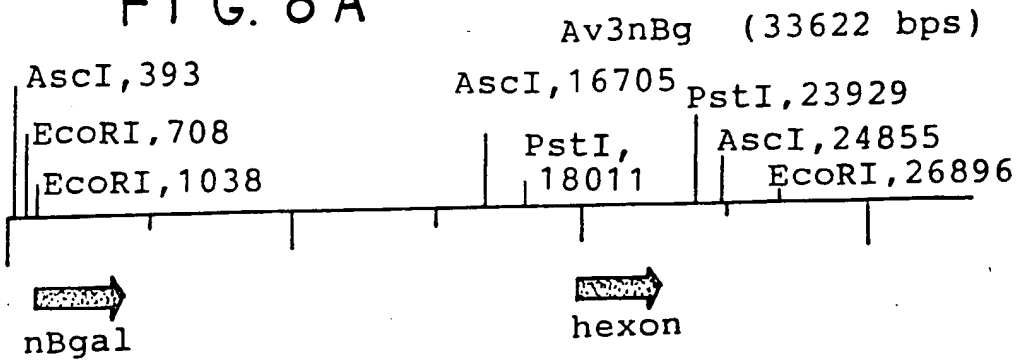


FIG. 8B

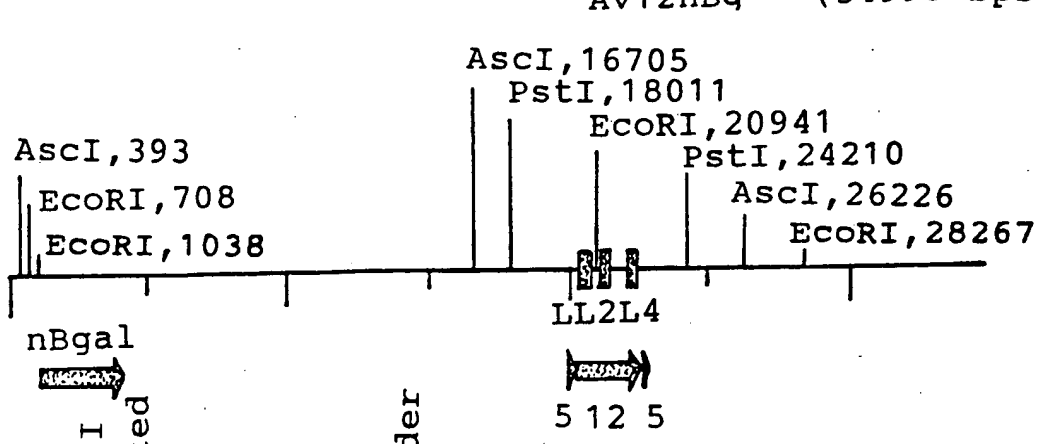


FIG. 8C

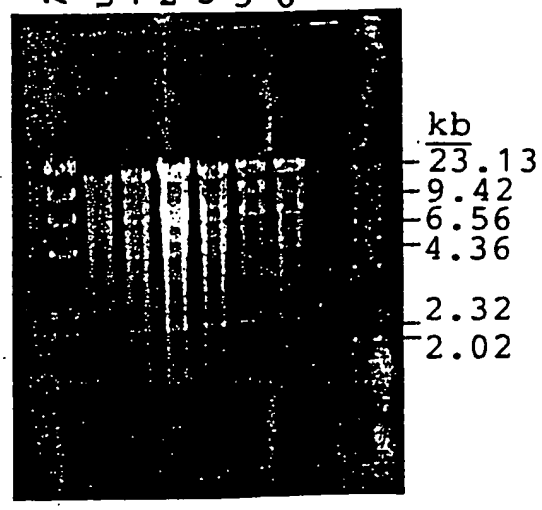


FIG. 8D

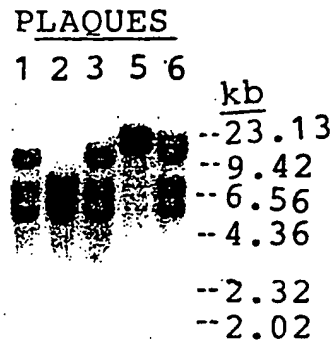


FIG. 9C

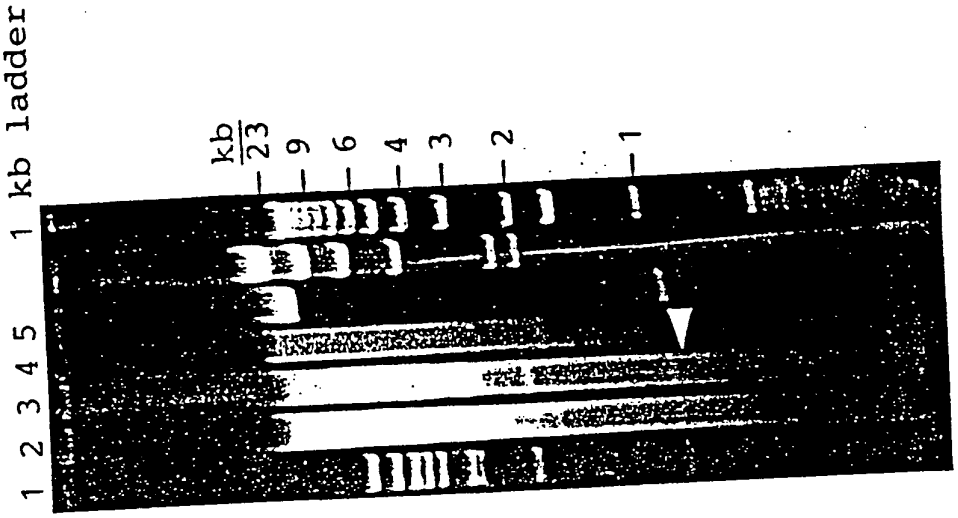


FIG. 9A

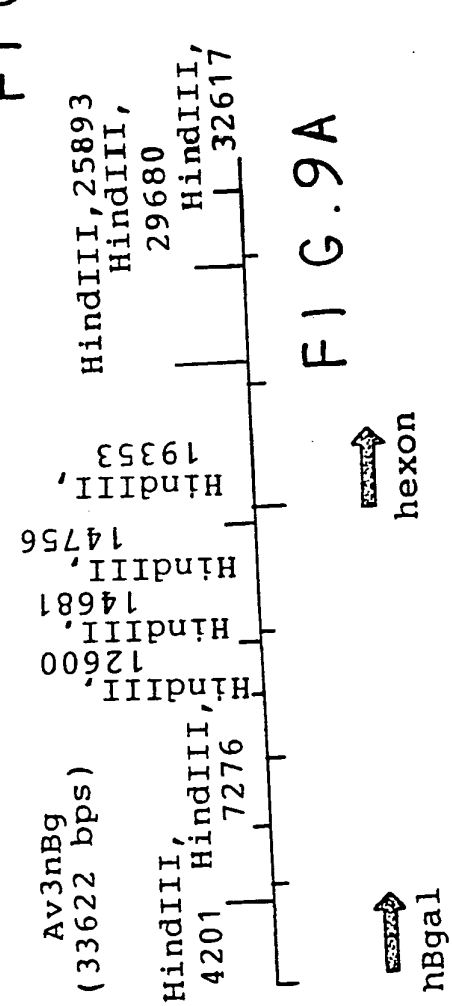


FIG. 9B

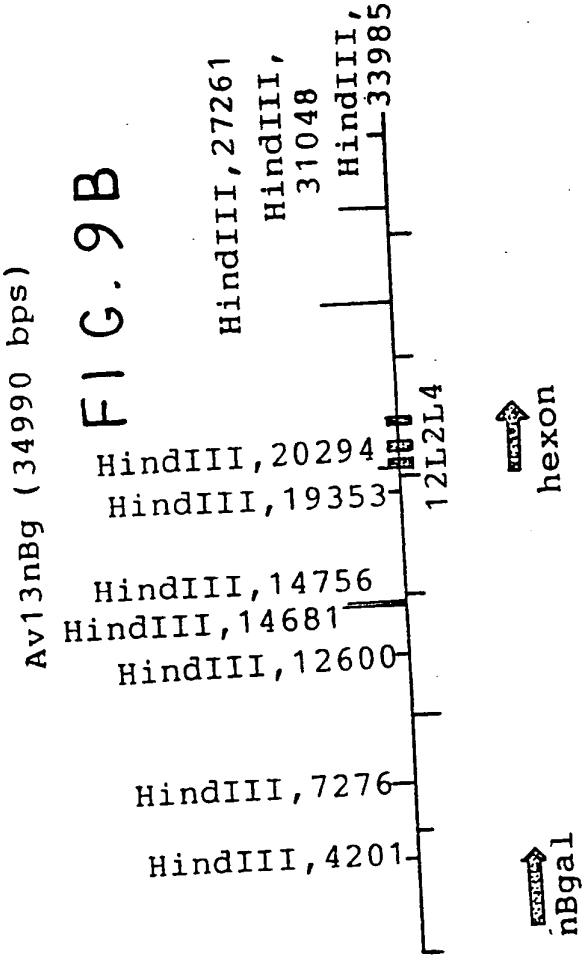


FIG. 10

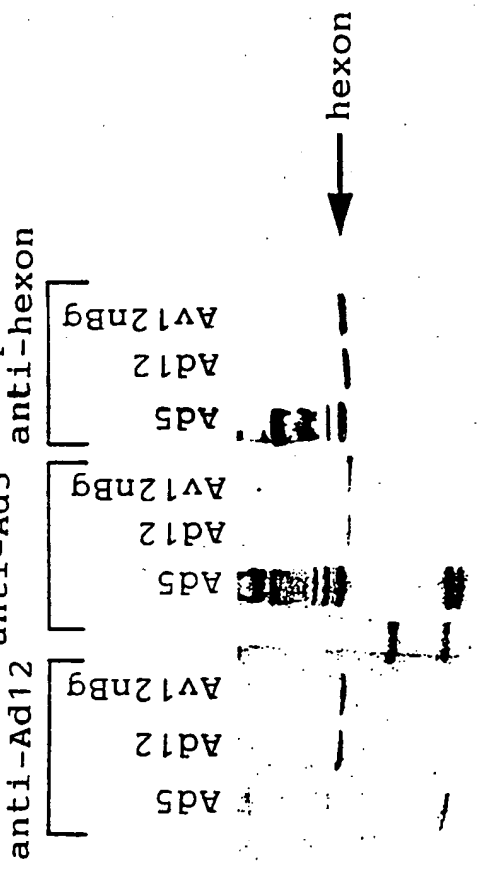

Antibody
anti-Ad12
anti-Ad5
pan anti-hexon
Sample
Ad5
Ad12
Av12nBg
hexon

FIG. 11

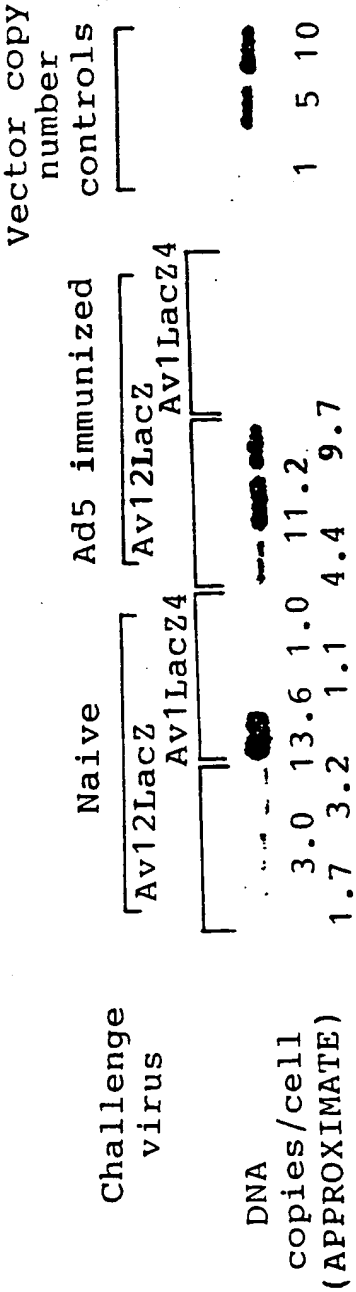

Challenge virus
Naive
Ad5 immunized
Av12LacZ
Av1LacZ4
Vector copy number controls
DNA copies/cell (APPROXIMATE)
1.7 3.2 3.0 13.6 1.1 1.0 4.4 11.2 9.7
1 5 10

FIG. 12A

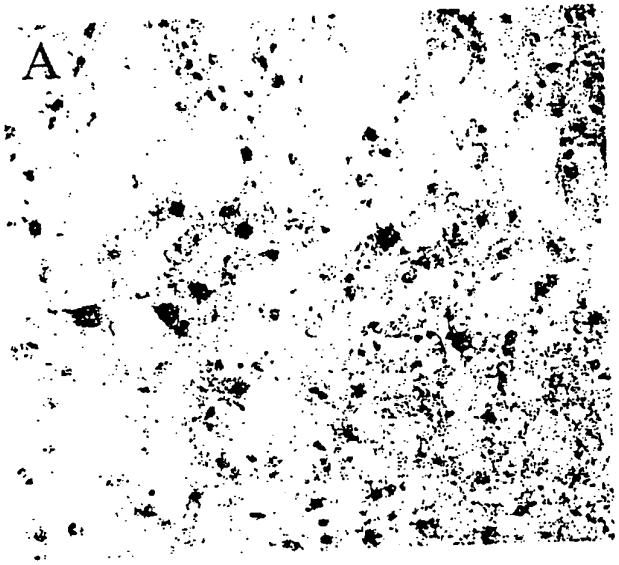


FIG. 12B

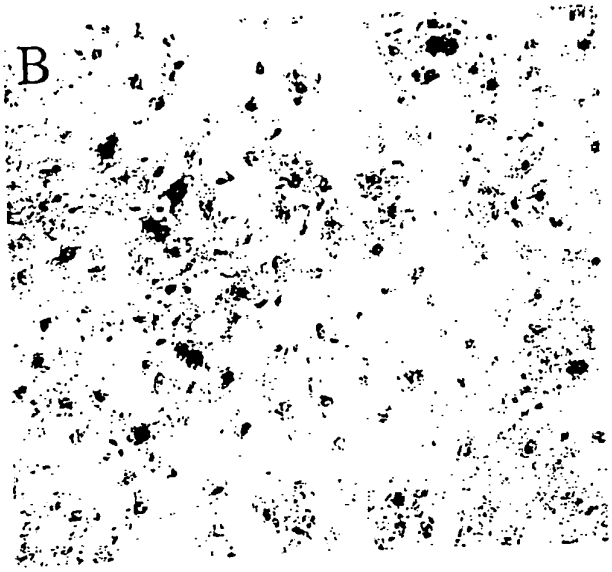


FIG. 12C

FIG. 12D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01113

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 7/01; A61K 35/76
US CL : 435/235.1, 320.1; 424/93.1, 199.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/235.1, 320.1; 424/93.1, 199.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, WORLD PATENTS, MEDLINE. SEARCH TERMS: ADENOVIRUS, ADENO VIRUS, ADENOVIRUSES, ADENO VIRUSES, HEXON#, SERO?, FIBER, LOOP, LOOPS, HYBRID, HYBRIDS, CHIMER?, CHIMAER?, RECOMB?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MASTRANGELI et al. "Sero-Switch" Adenovirus-Mediated In Vivo Gene Transfer: Circumvention of Anti-Adenovirus Humoral Immune Defenses Against Repeat Adenovirus Vector Administration by Changing the Adenovirus Serotype. Human Gene Therapy. 01 January 1996. Vol. 7, pages 79-87, see entire document.	1-10
Y	CRAWFORD-MIKSZA et al. Analysis of 15 Adenovirus Hexon Proteins Reveals the Location and Structure of Seven Hypervariable Regions Containing Serotype-Specific Residues. Journal of Virology. March 1996. Vol. 70, no. 3, pages 1836-1884, see entire document.	1-10

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date		
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search
11 MARCH 1998

Date of mailing of the international search report
05 MAY 1998

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Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/01113

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CROMPTON et al. Expression of a foreign epitope on the surface of the adenovirus hexon. Journal of General Virology. 1994. Vol. 75, pages 133-139, see entire document.	1-10
A	PRING-AKERBLOM et al. Sequence Characterization and Comparison of Human Adenovirus Subgenus B and E Hexons. Virology. 1995. Vol. 212, pages 232-236.	1-10
A	CRAWFORD-MIKSZA et al. Adenovirus Serotype Evolution Is Driven by Illegitimate Recombination in the Hypervariable Regions of the Hexon Protein. Virology. 1996. Vol. 224, pages 357-367.	1-10
A	MAUTNER et al. Recombination in Adenovirus: Analysis of Crossover Sites in Intertypic Overlap Recombinants. Virology. 1984. Vol. 139, pages 43-53.	1-100